CONTINENTAL CAN COMPANY, INC.

MOLD COUNTING

OF TOMATO PRODUCTS

By TROY (V.S.)

Mold Counting Tomato Products

Metal Division
esearch & Development Department
NENTAL CAN COMPANY, INC.

1960

Mold Counting of Tomato Products

By

V. S. TROY

METAL DIVISION

Research and Development Department

CONTINENTAL CAN COMPANY, INC.

Revised August, 1956

Reprinted May, 1960

TABLE OF CONTENTS

Mold Counting of Tomato Products

I. INTRODUCTION

IT IS ESTIMATED that over four million tons of tomatoes are grown annually in the United States, with more than three million tons being used for processing. (2) In addition to canned tomato juice, catsup and tomato sauce, large quantities of tomato puree and paste are used in the preparation of soups, pork and beans, spaghetti and numerous other items.

A large portion of the tomato products that are packed each year enter interstate commerce and are subject to federal Pure Food Laws. Many states have their own food laws as well. Regulatory officials have established very exacting standards for the purity and wholesomeness of our food products; and the preparation and manufacture of tomato products in accordance with existing standards is not simple. It requires intelligent direction and management that understands the causes for producing inferior products and the means of avoiding them. The Howard Mold Count on the finished product is one means by which the packer can check the efficiency of the operations—particularly the sorting and trimming of the tomatoes—employed in the preparation and canning of his tomato products.

The packing of acceptable canned tomato products is not restricted to any specific state or territory. It is true that some sections of the country produce greater quantities of fruits and vegetables than others by virtue of their climatic conditions or cultural practices. But in the main, the question of maintaining the wholesomeness and purity of the product rests with the individual canner. It is a matter of focus, direction, and effort. Growing, harvesting, and packing practices are not static and, over the years, we have seen a steady improvement that has come about largely through education.

II. THE SIGNIFICANCE OF MOLD IN TOMATO PRODUCTS

Mold growth, in significant quantities, is not found on sound tomatoes. The growth of mold on tomatoes breaks down the tomato tissue producing a rot. Therefore, the presence of mold filaments in tomato products is an indication of rot caused by previous damage. Federal and State Food Law enforcement authorities consider the amount of mold in canned tomato products to be an index of the care used by the packer—particularly in the sorting and trimming operations—to keep rot out of the canned product.

As increasing proportions of visible rot are allowed to remain on the tomatoes going to the extractor, the mold

count on the finished product increases, but the relationship is not an exact one due to the nature of the rot that is present. The character of the rotten portion varies greatly. Rots caused by some species of molds are soft and will be broken up in the extraction procedure. Other types of rot are hard and tough and will be largely discharged with the skins and cores without contributing much mold to the finished product.

Various investigators have studied the problem of the relationship of visible rot to mold counts. Eisenberg (3) and Smith (4) have published interesting articles on this subject. Howard and Stephenson (5), in pioneering work published in stated: "A low mold count do necessarily indicate sound stock high mold count always indica stock or improper handling." In handling refers to slimy tom cumulations on unclean equi Figure 1, reproduced from U. Bulletin 581 (5), summarizes averages the relationship of 1 terminations of visible rot to 25 counts on samples of tomat from 17 different factories on 3 Howard and Stephenson connec points on the graph which s minimum amounts of rot for a mold count and obtained a s

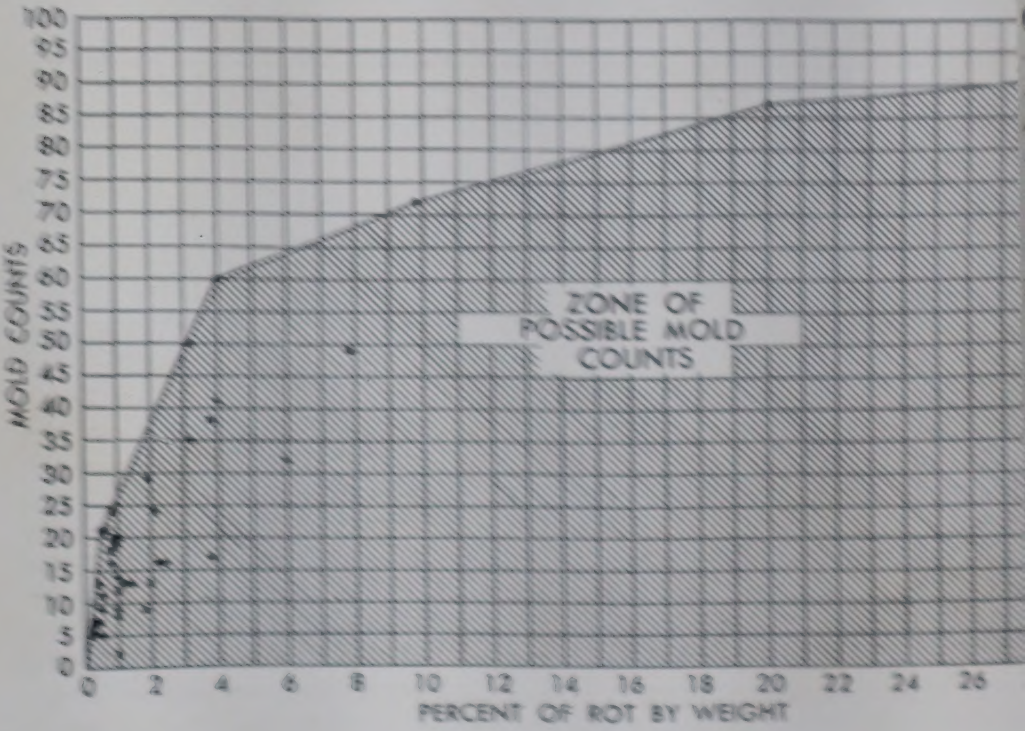


FIGURE 1

Relation between percentage by weight of cut-out rot and mold count.

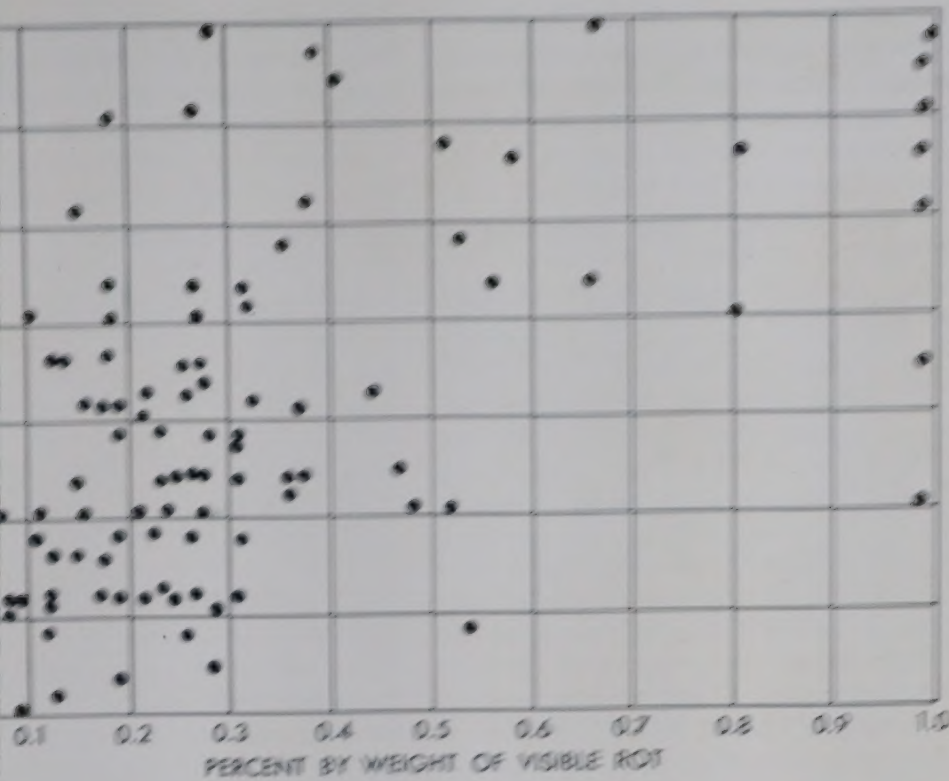


FIGURE 2

Relation of visible rot to mold count.

possible mold counts corre-
to a given amount of rot.
pparent that there would not
ual contribution of mold fila-
rom the various types and
of rot in proportion to the
of unfit material. On an
basis, therefore, these authors
d the mold count with the
n amount of rot present as
ed by the solid line connect-
outer points on the graph.

2, reproduced by N.C.A. In-
Letter No. 1371 (4), shows
form the relation of visible rot to mold counts on 124 samples of unhomogenized tomato products. Although the percent by weight of visible rot on tomatoes cannot be determined with precision, and the relation of visible rot to mold count has certain limitations, the packer should exercise every precaution particularly at the sorting belt, to prevent tomato rot from getting into the final product. The Food and Drug Administration regards high mold counts as indicative of unsatisfactory condition of the raw product used with respect to the presence of decomposed tomato material. Tomato products exceeding government mold

count tolerances are subject to seizure. When the significance of high mold counts is considered in terms of what they may mean to a packer in dollars and cents in case of seizures, t for maintaining careful sup over the preparation and cannir ations is readily recognized.

III. THE HOWARD MOLD COUNT METHOD

The Howard Mold Count Method was developed in 1910 by B. J. Howard of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, the federal agency then charged with the enforcement of the Food and Drug Act of 1906. Since the decay of tomatoes is largely caused by molds, Howard conceived the idea of using the occurrence of mold filaments in the comminuted finished product as a means of ascertaining the presence of decayed tomato material. Investigational studies were started in the fall of 1908 as a result of a conference between Howard and Dr. W. D. Bigelow, then chief of the Food Division of the Bureau of Chemistry. Studies were conducted by Howard in canning factories, and the official procedure was first described in U. S. Circular 68 (February 13, 1911) entitled, "Tomato Ketchup Under the Microscope with Practical Suggestions to Insure a Cleanly Product." (1)

The Howard Method was designed for two purposes: (1) to give the manufacturers a method for checking their product by determining if their sorters and trimmers were doing good work; and (2) to enable the federal Food Law Enforcement Agencies to prevent shipment in interstate commerce of tomato pulp or other strained tomato products which were made from moldy or decomposed material.

The method as now used re an improvement from its first tion in 1911 when an ordinary scopic slide was used and no designation was given for th filaments observed. The essenti ciple involved in the method unchanged since its first publ i. e. the correlation of a mic count of mold filaments wi amount of decomposition in the With improved instrumentatio ever, the technique of mold c has been standardized in order t greater uniformity in counting.

A.O.A.C. Official Method

The official A.O.A.C. met quoted below as it is given Official Methods of Analysis Association of Official Agric Chemists, (6) Eighth Edition page 781:

TOMATO PRODUCTS
(Not Dehydrated)

35.58 MOLDS (9)—OFFICIAL

"In making mold counts of products, use juice and catsup comes from container. In case o and paste add H_2O to make with total solid content that wi immersion refractometer reading of 45.0-48.7 or refractive in 20° of 1.3447-1.3460.

Steps in Mold Counting

It should be noted that the following details do not in any way change the Official Howard Mold Count Procedure.

(1) Cleaning of Microscope

The microscope is a delicate and expensive instrument and must be handled with extreme care. It is essential that all parts of the microscope be kept scrupulously clean, particularly the ocular, objective, and mirror in order to detect and correctly identify the mold filament, spores, yeasts and somatic cells that are present in the sample being examined. The glass surfaces should be wiped off with a soft cloth or clean chamois skin. If the dirt does not remove easily, breathe on the surface and rub gently.

(2) Standardization of Microscope

The microscope must be standardized with respect to the size of the microscope field. What most microscopists mean by the total diameter of the field can be set with the draw-tube. Binocular microscopes should be stan-

dardized at the optical company factory at the time of purchase. This important setting should be verified by checking against the calibration circle etched in the right shoulder of Howard Mold Count slides having a circular central disk or the two parallel lines etched in the oblong area of some makes of slides. The diameter of the circle, or the distance between the two parallel lines, is 1.382 mm as specified in the Official Method. When the microscope is properly adjusted, each field of view covers 1.5 sq. mm and the quantity of liquid examined per field is 0.15 cu. mm, which is equivalent to about 1/250th of a drop of sample.

The binocular microscope has two additional adjustments: one changes the distance between the eyepieces so that both eyes will see a single field. This is called the interpupillary adjustment. The centers of the lenses in the two eyepieces are adjusted until they are exactly the same distance apart as the centers of the observer's eyes. The other adjustment compensates for any difference between observers' eyes. To make this adjustment, the microscope is focused on a small object so as to be sharp to the right eye. Then the right eye is closed and the adjustment on the left eyepiece turned until the same object is equally sharp for the left eye. Then, both eyes will see the same object equally well.

(3) Preparation of Sample

The sample to be examined should be representative of the batch or lot from which it is taken. Juice and catsup are examined as they come from the container, without dilution. In case of puree and paste, water is added to make a mixture having a total solid content that will give an immersion refractometer reading at 20° C. (68° F.) of 45.0-48.7 or an index of refraction at 20° C. of 1.3447-1.3460. This is equivalent to total solids as determined by drying in vacuo at 70° C. (158° F.) of 8.5% (specific gravity 1.0353) to 9.45% (specific gravity 1.0394). In a tomato product plant, the analyst knows the approximate concentration of the product with which he is working and dilution may be made according to Table 6 of N.C.A. Bulletin No. 27-L (Tomato Products, Rev.). (7) Examples of these dilutions are shown in Table 1. Products of higher concentration than the range of Table 6 (7) may first be diluted with water until they come within that range and the dilution handled according to the table. Tomato paste is diluted 1:2 of paste to water. The sample taken for examination must be thoroughly mixed before the preparation of each slide. This may be accomplished by pouring the contents from one container to another several times.

(4) Cleaning of Howard Slide and Cover Glass

The slide and cover glass may be cleaned with water and a dry, lint free cloth. It is suggested that the cleaned cover glass be placed on one end of the slide until used to keep it free from dust or dirt. Do not lay it on the table. A test for cleanliness of the cell is to place the cover glass in position and press it firmly against the shoulders. If Newton rings appear between each shoulder and the cover glass and

TABLE 1

DILUTION OF PUREE (PULP) FOR MOLD COUNT

Actual Sp. Gr.	*Actual Ref. Index*	*Desired Sp. Gr.*	*Desired Ref. Index*	*Amt. of Water to be added to 100 ml. of Sample*	*Total Volume of Diluted Sample*
1.040	1.3462	1.035	1.3446	14.5 ml.	114.5 ml.
1.045	1.3478	1.035	1.3446	29.2 ml.	129.2 ml.
1.050	1.3494	1.035	1.3446	44.0 ml.	144.0 ml.
1.055	1.3511	1.035	1.3446	58.5 ml.	158.5 ml.
1.060	1.3527	1.035	1.3446	73.0 ml.	173.0 ml.

remain after the pressure has been released, the cell is considered to be sufficiently clean. These rings resemble a rainbow in color and form broken arcs of concentric rings. They may be observed by holding the slide at such an angle that the light is reflected from the cover glass.

(5) Preparation of Slide

After the slide is thoroughly cleaned, a small drop of the well-mixed sample is placed upon the central disk. This is accomplished by dipping a clean scalpel (preferably plastic to avoid scratching the glass) into the sample, and with a scooping motion, obtain the desired amount of product so that when it is transferred to the central disk, the quantity will be sufficient to entirely cover the disk but with only a slight amount of sample squeezing over the edge of the disk. The use of a dissecting needle in removing the sample from the scalpel to the central disk may be helpful particularly with products of heavy consistency such as catsup and puree to insure getting the proper proportion of soluble and insoluble tomato solids.

The "sample drop" is spread evenly over the disk using the scalpel. The cover glass is then held in a slanting position with one edge resting on the two shoulders and lowered to a point where it is almost touching the sample on the disk, then it is lowered rapidly into place to spread the sample evenly over the entire disk. The cover glass should not be lowered too quickly, otherwise it will cause part of the sample to splash over onto one or both shoulders, thus ruining the mount. If the cover glass is lowered too slowly, the sample will not spread evenly over the disk, and the insoluble material (and consequently mold filaments) may be more abundant at the center of the mount.

The preparation of satisfactory slides showing even distribution of insoluble material will come with practice. Discard any mount showing uneven distribution or absence of Newton rings or liquid that has been drawn across

the moat and on to the shoulders of the slide. Care should be taken to avoid entrapping air bubbles. An analyst should never count a slide with which he is not entirely satisfied.

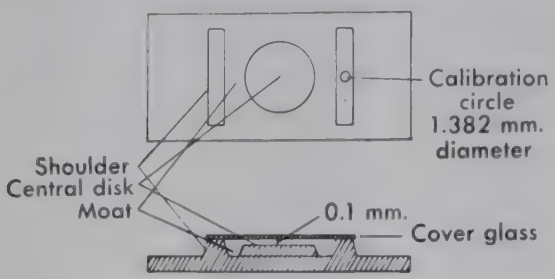


FIGURE 3

Howard Mold Counting Chamber.

(6) Adjustment of Illumination

Proper illumination is essential. Too much light will conceal fine molds and too little light will not reveal the mold distinctly. The light can be adjusted properly for each particular field by focusing carefully on a filament and then opening or closing the iris diaphragm or raising or lowering the condenser to produce maximum distinctness for the analyst.

(7) Counting of Sample

The slide is placed on the microscope stage using approximately 100X magnification (10X ocular and 10X 16 mm. objective) with such adjustment that each field of view covers 1.5 sq. mm. The official method states "from each of two or more mounts, examine at lease 25 fields taken in such manner as to be representative of all sections of mount." (6) (A microscopic field is the value of material in the circular field of view observed on the central disk when examined under approximately 100X magnification. Each time the slide is moved a new "field" comes into view.) This is usually accomplished by counting alternate fields, skipping every other field, as the slide is moved horizontally and skipping every other row as the slide is moved vertically. The ideal distribution of the fields counted would then be arranged as shown in Figure 4. With a little practice, this method of field selection can be very closely approximated.

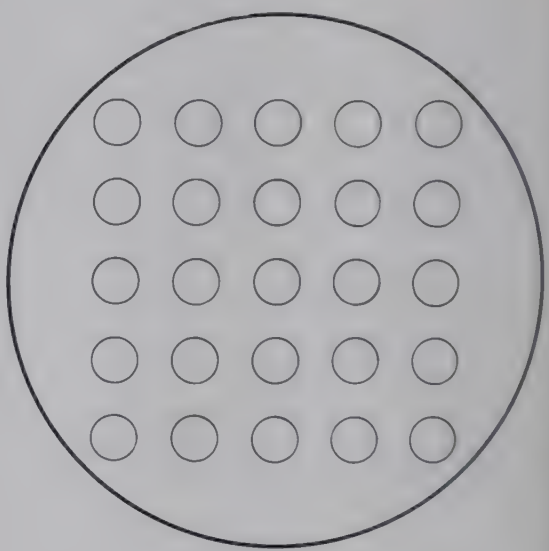


FIGURE 4

Illustration of ideal field selection.

Each field should be carefully examined noting the presence or absence of mold filaments and recording the results as positive or negative as the case may be. In those few instances where identifying characteristics are not clearly discernible, use magnification of approximately 200X (8 mm. objective) to confirm identity of the

filament. The fine adjustment knob is always used to bring into view filaments that may be at different depths in the field. No field should be considered positive, unless the aggregate length of not more than three of the mold filaments present exceeds 1/6 of the diameter of the field. The intensity of illumination may require changing to give proper inspection to fields of different density. Any doubtful filament should be brought to the center of the field for better visibility and identification and then returned to its original position. The analyst, while looking through the microscope, should change the focus continually using the fine adjustment so as to show the finer detail of mold filaments.

It is essential that the fields be selected systematically without reference to the presence of filaments and the analyst should use a definite procedure for examining every portion of the field. If a field shows the presence of a large amount of mold, the analyst should record the field as positive and quickly move to the next field to be counted. If a field is negative, or shows upon first glance only a small amount of mold, a detailed search of every part of the field is necessary. It should be pointed out that negative fields are just as important as positive fields. In estimating the length of the mold filaments to score a field positive, one filament is usually sufficient. However, occasionally two filaments are required and rarely, the aggregate length of three filaments is needed. A special drop-in micrometer disc inserted in the ocular of the microscope divides the field into squares, each side of which is 1/6 of the diameter of the field and aids materially in estimating the length of the filaments.

(8) Calculating Results

"From each of 2 or more mounts examine at least 25 fields taken in such a manner as to be representative of all sections of mount. Calculate proportion of positive fields from results of examination of all observed fields and report as percentage of fields containing mold filaments." (6) The percent of positive fields can be calculated by use of the following formula:

$$\frac{\text{Number of positive fields}}{\text{Number of fields examined}} \times 100 = \%\ \text{positive fields}$$

Example: No. of positive fields 4, 4 = 8 (2 slides)
No. of fields examined 50 (2 slides)

$$\frac{8}{50} \times 100 = 16\%\ \text{positive fields}$$

Example: No. of positive fields 6, 10, 8 = 24 (3 slides)
No. of fields examined 75 (3 slides)

$$\frac{24}{75} \times 100 = 32\%\ \text{positive fields}$$

Interpretation of the Mold Count Results

In order to obtain reliable results, thorough mixing of the sample, the preparation of the slide, and the counting of the slides must all be done with the utmost care in accordance with the official method.

The results on a single slide are of limited value. More than one slide should always be counted. It has been suggested, as a working basis, that two slides must check within three positive fields of each other, otherwise, one or more additional slides must be counted.

The Howard Mold Count has been carried out in essentially the same manner since it was originated in 1910. A large amount of data have been accumulated through the years with respect to the significance of mold counts in terms of the character of the raw product and the methods used in the washing, sorting and trimming operations. That the method does have limitations is well recognized and these limitations are taken into consideration in the interpretation of the results. Many different kinds of molds are present on tomatoes as grown and as received at the factory. Fortunately, in making official mold counts, no attempt is made to differentiate between types of mold which may be present. The method is based upon the common characteristics of the filaments which are to be classified as mold.

The validity of the Howard Method has been challenged many times over the past years, but it has been upheld in numerous court trials. Although natural variation occurs in the method, this variation can be controlled by statistical sampling techniques. These would include the examination of additional samples from the same code and counting more fields on each sample; in other words, applying statistical principles to the design of a particular lot being examined. Competent analysts will obtain substantially the same results. The method has been used by the tomato industry for over 40 years. It is now judicially recognized as the official method of determining the presence of mold in tomato products and, on the basis of the counts obtained, as an index of the amount of decayed tomato material in comminuted tomato products. The Howard Method is empirical and must be followed exactly as given in order to obtain satisfactory results. Only those counts made in accordance with the official procedure can be interpreted by the manufacturer or by regulatory agencies.

Normal Variations in Mold Counting

Any discussion about the variations in mold counts obtained on different slides should keep in mind two ideas which are separate and entirely distinct. The first is that the identification of the mold filaments in every field must be considered correct. The second consideration regarding the nature of tomato products with respect to the distribution of the mold filaments has to do with the interpretation of the analytical results. As seen by the analyst, tomato products consist of a mixture of tomato tissues and mold filaments. Therefore, we must

consider the limitations of distribution as they apply to the sampling of any such mixture. A paper which includes a discussion of this subject was presented by Dr. W. D. Bigelow (8) before the tomato and tomato products section of the National Canners Association in 1933. The data reported was based on a study of mold counts on tomato products made by analysts in the Washington and San Francisco laboratories of the N.C.A. in which six to twenty-seven slides were examined from each of 78 samples. The following statement is quoted from this article: "The mold filaments are mixed with the insoluble tomato structures of the sample. It is well-known that a mixture is never uniform to the extent that every sample taken from a thoroughly mixed product should be expected to show just the true proportion of each constituent. Most of such samples will show close to the true proportion but some will be further away. This grouping of the results obtained on individual samples about the average or true results has been investigated in a large number and variety of mixtures and the same distinct trend has been found in each case. Certain formulae and rules have been adapted to these experiments by the use of which it is possible to state the likelihood that a single result will not differ from the average by more than a certain amount." (8) An important contribution to this subject was made by Dr. J. D. Wildman of the Microanalytical Laboratory of the U. S. Food and Drug Administration. (9) Dr. Wildman found that microscopic counts follow those same tendencies. He reported the results of a series of microscopic counts on each of three mixtures. A study of the results showed that they are grouped about the average in the same way that had been observed in connection with other mixtures and the same rules and formulae could be applied. The microscopical examination of a portion of a drop of mixture of very small substances is as accurate as the examination of mixtures of larger substances by any of the usual methods.

With the statistical explanation, it is now possible to reassure competent analysts that occasional wide variations in counts may well be accounted for solely on the basis of the distribution of the filaments, and that a properly trained analyst is not to change his method of examination because of such variations in individual slides. This statistical explanation is very reassuring. It proves that the mold count method itself is valid because the results are in accord with the general experience of sampling difficulties with all mixtures. If we did not have such difficulties, it would indicate that the method was abnormal and therefore subject to criticism.

Data obtained by the N.C.A. as reported by Smith (10) indicates that if the counting is done correctly at every step and if the true count of the sample is 50 percent positive fields, successive individual slides will normally be expected to indicate between 43 and 57 percent positive fields in two-thirds of the slides examined, but the other one-third of the slides examined may have positive fields indicating that the sample contains less than 43 percent or more than 57 percent positive fields. These greater variations are due entirely to the normal distribution of

the mold filaments and have nothing to do with the ability of the analyst or the additional uncertainties of the preparation of the slide. Fortunately, the most probable results are those close to the average and the variations from the average tend to compensate each other. There is no way to avoid these variations. Only by the examination of a larger number of slides will the average of all results obtained come nearer to the true count.

Confirmation of Distribution by Analytical Method

A question often asked is, "How can the examination of a fraction of a drop of tomato catsup indicate the purity of an entire batch of catsup?" This is a reasonable inquiry.

Recently a procedure was developed by Smith (11) for providing large known numbers of imitation mold filaments which can be mixed with a known volume of tomato product to give a sample containing a predetermined proportion of mold-like filaments of microscopic size. The examination of such a known sample has yielded results which give information on the reliability of the Howard Mold Count Method. Smith concluded that "known numbers of microscopic fragments resembling mold when mixed with tomato products can be discovered and the approximate number determined quantitatively with a satisfactory degree of accuracy. The filaments are distributed at random throughout the sample as shown by the general relationship of the results on individual slides to the results predicted by the binomial distribution. The number of slides that must be examined to attain any desired degree of accuracy for the average may be calculated from the general considerations of random distribution." (11)

The answer then to the original question is that "all of the mold filaments from the rot are distributed at random throughout the entire batch and each minute part of every drop has its proportion of the filaments. The filaments can be found, identified and measured by the official Howard Mold Count Method. Although the product is a mixture, which makes sampling difficult, the average of several slides will be near the actual proportion." (11)

Interpretation of mold count results requires a knowledge of tomato products and a basic understanding of the statistical significance of the random distribution of the mold filaments.

IV. TECHNICIANS SCHOOLS FOR INSTRUCTION IN MOLD COUNTING OF TOMATO PRODUCTS

The Howard Method for determining the mold count on tomato products cannot be mastered without personal instruction in the details of the method. One of the best ways for training analysts is to have them attend one of the several technicians mold count schools throughout the country. The schools are sponsored by State Canners Associations in conjunction with the N.C.A. and usually extend over an eight-day period.

Packers of tomato products employ analysts during the canning season to check their products as they are being manufactured. Since this is seasonal work, many new analysts must be employed each year and the instruction of these analysts in the details of the method is a regular part of the preparation for the canning season. Many of these analysts have not had previous training or experience in using the microscope; others have had years of experience in this type of work but return each year to the school for a day or two which they consider as a "refresher course."

The course of instruction given at the Technicians Schools consists usually of a series of lectures, demonstrations, and special studies. These include the following:

1. The construction, care and use of the compound microscope.

2. The histology of the tomato.

3. The characteristics of the mold filaments.

4. The construction and use of the special Howard Mold Count Cell.

5. The details of technique of the official Howard Mold Count Method.

6. Special studies on the identification of mold filaments.

7. Special laboratory interest in the nature of the product being examined.

8. Interpretation of the results.

The first mold counting school was held at the National Canners Association in Washington, D. C. in 1915, under the supervision of B. J. Howard of the U.S.D.A., and instruction for employees of the N.C.A. member canners has been continued each year since. The Western Branch Laboratories of the N.C.A. also have provided instruction in the Howard Mold Count Method for the employees of member canners since 1926. The N.C.A. is in charge of instruction at the schools sponsored by the various state canners associations, and is assisted by representatives of the research departments of the major can manufacturing companies.

Technicians Schools for instruction in mold counting of tomato products have been conducted at Purdue University, Lafayette, Indiana, since 1935 and at the New York State Agricultural Experiment Station, Geneva, New York, since 1937. Similar schools have also been sponsored by the state canners associations of Arkansas, Louisiana, Maryland, Texas, Utah, and in the provinces of Ontario, Quebec, and British Columbia in Canada. Similar Technicians Schools for instruction in mold counting of pineapple juice have been conducted in Puerto Rico and Mexico.

Since the inception of the Technicians Mold Counting Schools, many hundreds of analysts have learned the

method and have been carrying it out in accordance with the official procedure. As new analysts must be trained each year and since the details of the method are very exacting, it seems that these schools will continue to serve a very useful purpose in the tomato products industry.

V. THE HISTOLOGY OF THE TOMATO

Proficiency in mold counting requires that the analyst identify every particle in each field as part of the tomato or normal constituent of tomato product or mold filaments.

In the process of manufacture, tomatoes pass through finishers or screens and the cells are broken into small pieces making them, at times, very difficult to differentiate from some mold filaments. A competent analyst must, therefore, be very familiar with the histology of the tomato. It is as important to know what is not mold as what is mold. The tomato cells and fibers most commonly encountered are shown in Figure 5.

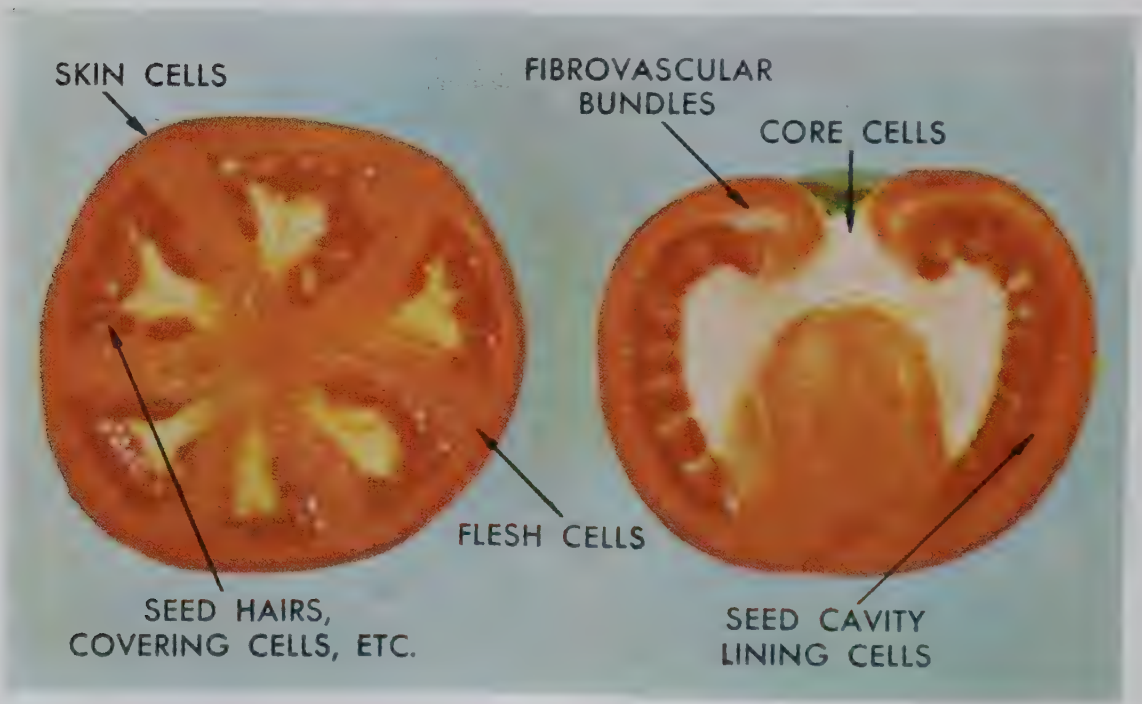


FIGURE 5

Tomato sections showing location of cells and tissues.

Skin cells are recognized by their greenish yellow color and irregular characteristic shape. Although separate cells, they fit closely together and show definite cell wall outlines. The cell contents have a light brown cast. Under 100X magnification, a piece of skin closely resembles the surface of hammered aluminum.

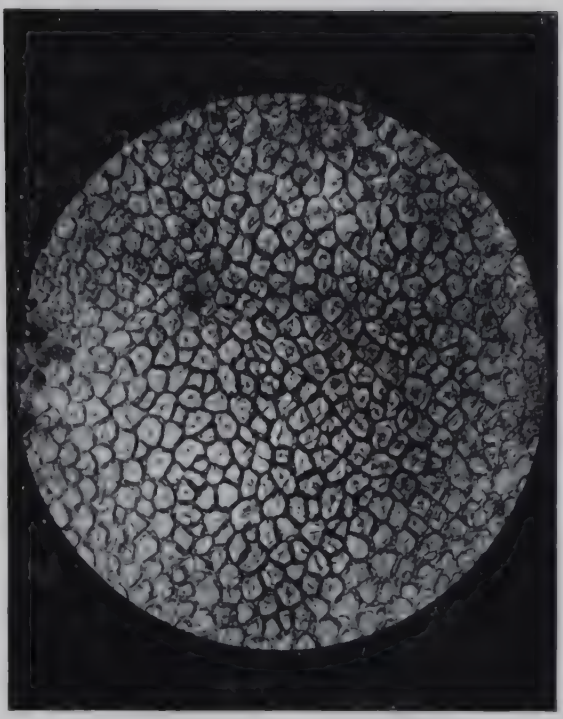


FIGURE 6

Epidermal or skin cells (100X).

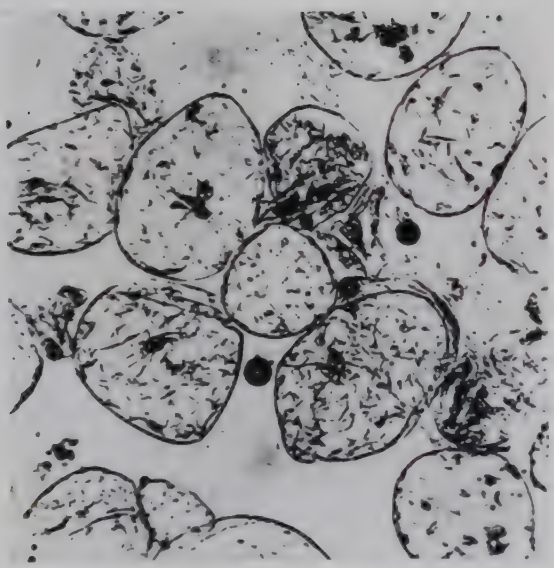


FIGURE 7

Flesh Cells (100X).

The flesh cells are large and very thin walled. They vary in shape from oval to circular and are often referred to as "cellophane footballs." The cells lying just beneath the skin and next to the edge of the seed cavity are smaller and narrower. The cell contents are sparsely granular and appear to be made up of tapering lines which in reality are folds in the cell wall.

The fibrovascular tissues are the white, thread-like veins in the tomato that carry moisture and nutriment throughout the fruit. Under the microscope they are dark in color and the vascular elements of the bundles resemble coiled springs in appearance. In comminuted tomato products the bundles are often broken and release short pieces of "springs," many of which resemble the letter "S". These broken vascular cells are often confusing to an analyst. However, they may be recognized by their uniform curling. They are also usually pointed or have a loop on one end.

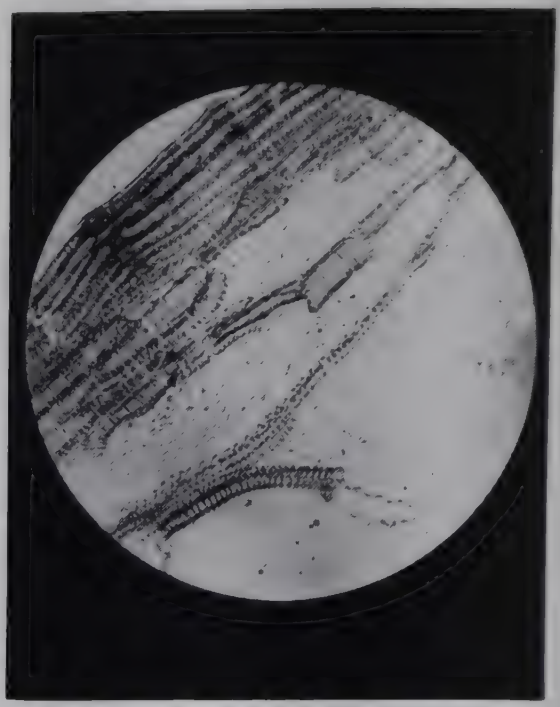


FIGURE 8

Fibrovascular Tissues (200X).

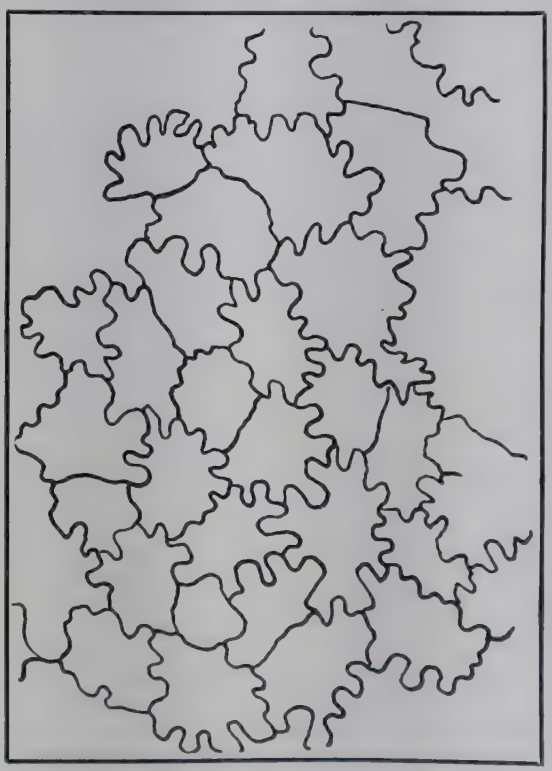


FIGURE 9

Seed cavity lining cells (100X).

The cells lining the seed cavity are rather small and thin walled. They are irregular in shape resembling pieces of a jig-saw puzzle in appearance. The cell contents are almost clear except for occasional flecks of cellular material.

The seed coat is covered with small hairs. They are usually attached but occasionally may be found singly. They are thin walled, long, tapering structures with a bluish tinge and, unless broken off, are pointed. Singly, their appearance resembles an icicle. The cell contents are almost colorless. The seed covering cells are very small and the outline of the cell is indefinite. The cell walls are thin and tend to arrange themselves into various patterns, the most common of which is a many pointed "star-like" structure. These cells make up the skin to which the seed hairs are attached.

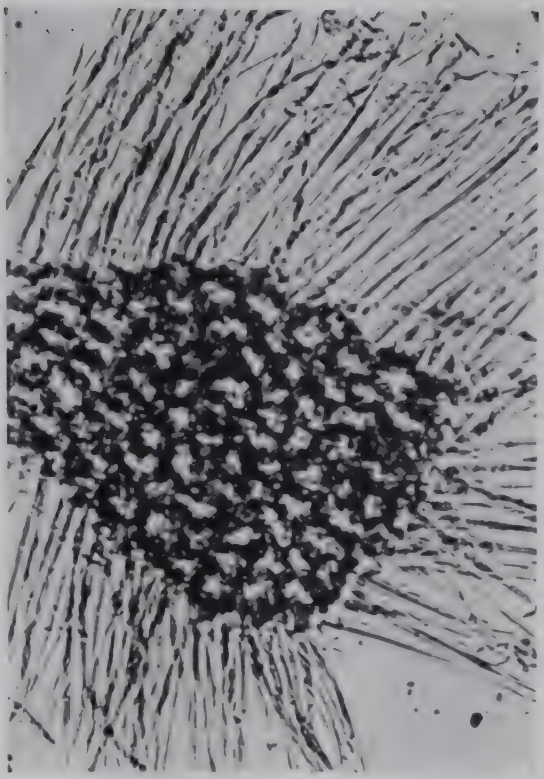


FIGURE 10

Seed hairs and seed covering cells (100X).

FIGURE 11

Internal seed cells (100X).

The internal seed cells vary widely in size and appearance. They range in shape from brick-like rectangular to nearly square cells, the square cells being several times larger than the more compressed brick-shaped cells. The cell content of the large, square cells is nearly clear, while that of the more rectangular cells is darker and finely granular.

The core cells are small and round with rather thick walls. The contents of the larger cells are rather clear while the smaller cells have a light amber color due to the more dense cellular materials. The larger cells tend to occur in clumps surrounded by many small cells.

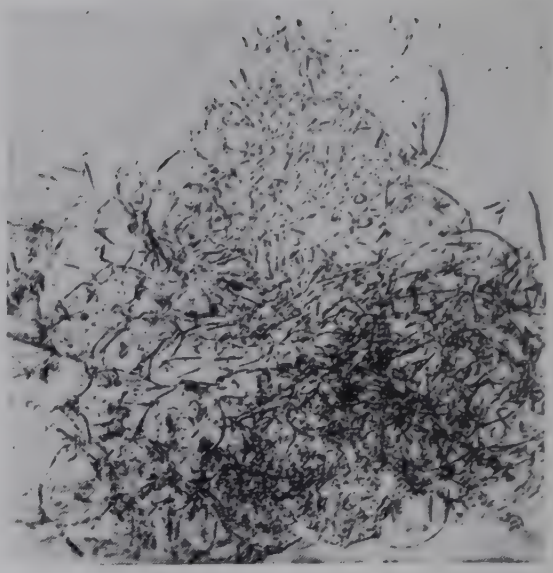


FIGURE 12

Core cells (100X).

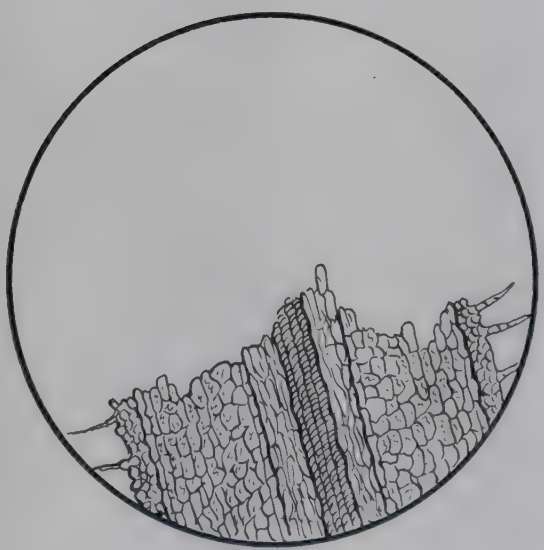


FIGURE 13

Stem cells showing epidermal hairs. (100X)

The stem cells vary in shape and size depending upon their location in the stem; however, they are usually rectangular in appearance. In relation to the flesh cells of the fruit, the stem cells are rather small. They are characterized mainly by the presence of chlorophyll, a green coloration within the cell. The epidermal hairs of the stem are long and tapering and are usually divided into three segments.

VI. CHARACTERISTICS OF MOLD

The characteristics for the identification of mold hyphae in tomato products are described in Food and Drug Circular No. 1, "Microanalysis of Food and Drug Products," issued by the Federal Security Agency. (12) They are described as follows:

Identification of Mold Hyphae

"Hyphae as a general rule occur in comminuted foods unaccompanied by fruiting bodies. Since the identification of specific molds is based for the most part on the type of fruiting body produced, it is generally impossible to determine from the hyphae the kind of mold present. However, it is important that the analyst be able to differentiate between mold hyphae and the normal elements found in the product. If the counting is on a comminuted fruit or vegetable product, he should assure himself, by microscopical examination, of the basic fact that the rot itself consists of the fruit tissue and mold. The analyst should examine the mold hyphae and determine those features whereby he can identify the hyphal fragments and clumps when the fruit is pulped. Mold hyphae in all cases are tubular although they may appear to be flat under the microscope. In most instances the diameters of tubes are uniform, and hence the cell walls appear under the microscope as parallel lines. Two conspicuous exceptions are the molds of the Mucor type and the Oospora, where the hyphae are often tapering." (12)

Granulation

"Growing molds have living protoplasm within the tubular structure. In the growing portions of the mold, the protoplasm may fill the entire space or it may surround vacuoles of cell sap. In either case, the protoplasm usually presents a granular or stippled appearance. This character may persist after the mold is killed in processing, or the protoplasm may coagulate into nongranular hyaline masses or plugs within the mold hyphae, leaving a considerable length of the tube free from protoplasmic material and thus empty in appearance.

"Granulation is prominent in the Mucor and Rhizopus molds and may be indistinct in others. There may be only traces of protoplasmic material in many filaments of the molds frequently found in butter." (12)

Septation

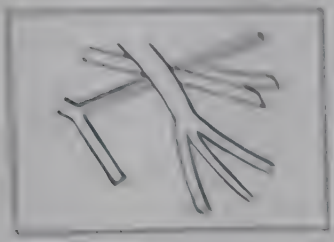
"Most molds encountered in foods contain cross walls. These may be thought of as walls separating the tubes into sections. Such molds are spoken of as being septate. The presence of cross walls may serve to positively identify otherwise doubtful filaments. However, cross walls are generally absent in Mucor mold." (12)

Branching

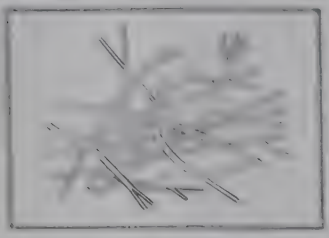
"Most molds show an abundance of branching. Branching is frequently an aid in the positive identification of mold, although often fragments of mold are too short to show branching." (12)

An article by Smith (10) gives additional suggestions to assist the microanalyst in the identification of mold filaments. In order to overcome uncertainty in identification of questionable filaments, Smith suggests the following rules:

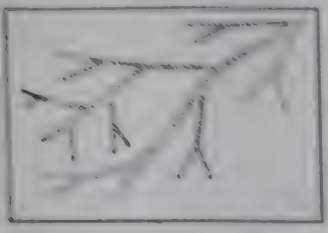
Only filaments which have at least one of the following characteristics shall be classified as mold hyphae:



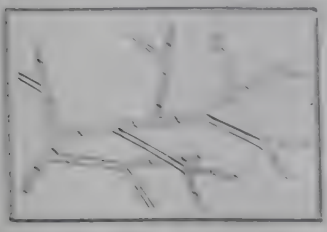
(1) Parallel walls of even intensity with both ends definitely blunt.



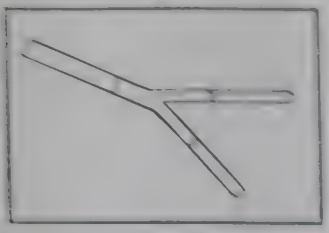
(2) Parallel walls of even intensity with characteristic branching.



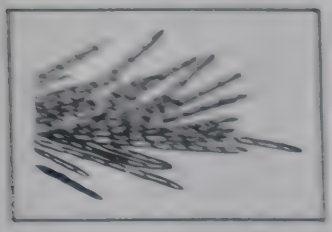
(3) Parallel walls of even intensity with characteristic granulation.



(4) Parallel walls of even intensity with definite septation.



(5) Occasionally encountered, parallel walls of even intensity with one end blunt and the other end rounded.



(6) Occasionally encountered, slowly tapering walls of even intensity with characteristic granulation or septation.

Mold filaments include both fertile d vegetative hyphae. The fertile phae are those which bear the fruit- e bodies or reproductive spores and ow above the surface. These are moved by efficient washing of the tomatoes, and are seldom seen in commercially canned products. The vegetative hyphae grow below the surface of the tomato and are the ones with which the analyst is primarily concerned.

VII. PRINCIPAL MOLDS ENCOUNTERED IN TOMATO PRODUCTS

Rot in tomato products usually results from the growth of molds and her microorganisms which have ined entrance into the tomato rough some defect in the skin. A otable exception to this is the Antomoz which may penetrate the ibroken skin of a normal tomato. ose skin defects may be caused by cessive rains which are particularly amaging to the fruit at harvest time nce they may cause the fruit to crack en or to unbalanced moisture conitions which produce cracks in the omatoes and thus allow the entrance f molds and other forms of microorganisms. Tomatoes resting on the soil ften begin to rot at the point of ontact. Any one of a number of olds may cause the rotting once has gained entrance into the mato.

Fortunately, in making official mold counts no attempt is made to differentiate between the types of mold which may be present. The Howard Method is based on the common characteristics of the filaments of molds usually encountered in tomato products. A description of the principal molds is given in Food and Drug Circular No. 1, "Microanalysis of Food and Drug Products" (12) issued by the Federal Security Agency; by Howard (13) in his publication, "Outlines for Instruction in Tomato Microscopical Methods," and in the U.S.D.A. Agriculture Handbook No. 28, "Market Diseases of Tomatoes, Peppers and Eggplants" by Ramsey, *et al.* (14)

Some of the principal molds found on tomatoes which may be observed in comminuted tomato products are listed on the following pages:

FIGURE 14

Alternaria tenuis (Alternaria Rot)

FIGURE 15

Alternaria tenuis (Alternaria Rot)

FIGURE 16

Alternaria solani (Alternaria Rot)

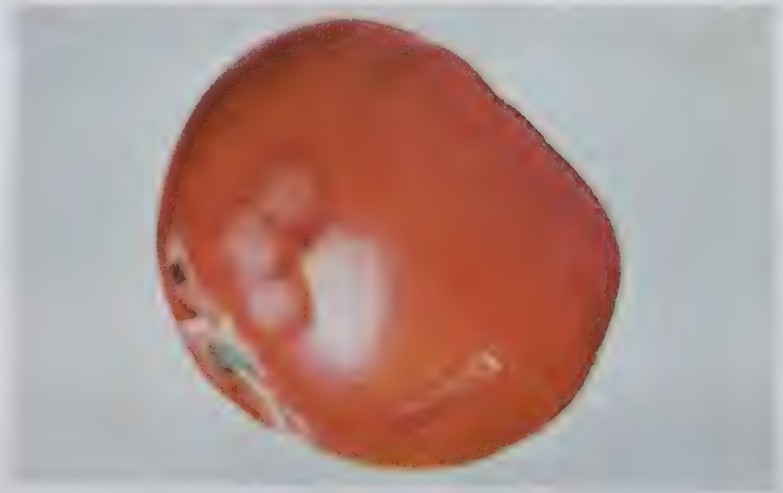


FIGURE 17

Colletotrichum phomoides (Anthracnose Rot)

FIGURE 18

Oospora lactis f. parasitica (Watery Rot)

FIGURE 19

Rhizopus nigricans
(Rhizopus Rot)

FIGURE 20

Phytophthora infestans
(Late Blight Rot)

Alternaria Rot

Alternaria is one of the most prevalent forms of molds that produce decay on the tomato in the field. It usually causes a darkening of the tissues, producing a "black rot." The most prevalent type of alternaria rot which the canner will encounter is that caused by *Alternaria tenuis*. *Alternaria tenuis* is a weak pathogen that attacks only injured or weakened tissues such as that caused by sun scald, blossom-end rot, growth cracks, or faulty blossom scars. Tomatoes harvested near the end of the season from weakened vines are particularly subject to alternaria rot. (Fig. 14 and 15) Another species, *Alternaria solani,* causes "Early Blight Rot." (Fig. 16), a small, leathery spot with zoned markings. This spot is much less prevalent than that caused by *A. tenuis*. The filaments of *Alternaria* are septate and of medium thickness. The spores are typical Indian-club shaped. (Fig. 21 and 22)

Anthracnose Rot

Anthracnose rot is caused by the fungus *Colletotrichum phomoides* and is one of the worst forms of decay the tomato packer encounters, as the anthracnose spots usually appear on several sides of the tomato, making it impractical to trim; thus it is necessary to discard the entire tomato. This fungus is believed to live over from season to season on plant debris in the soil and in and on the tomato seed. Warm wet weather favors spread of the fungus.

The production of anthracnose rot by the mold, *Colletotrichum phomoides* is unusual, in that it is not dependent upon a defect in the fruit in order to gain entrance. The spores usually germinate on the surface of the green tomato and, as the fruit ripens and the tissues become softer, the mold penetrates the apparently normal, unimpaired skin of the tomato. The growth is characterized by a concentration of the mycelia and spores just beneath the surface of the skin. In the early stage, anthracnose lesions are small, circular, slightly sunken, water-soaked spots. When the spots attain a diameter of about ½ inch they become darker, and cream to salmon-pink spore masses appear in the center. Later these spore masses appear as dark dots arranged more or less in concentric rings. The mycelium is septate and very fine, having the smallest diameter of any of the molds commonly infesting tomatoes. The spores are small and elliptical in shape. Anthracnose rot often causes high mold counts in comminuted tomato products. (Fig. 17, 23 and 24)

Oospora (Oidium) Rot

This genus is referred to as *Oospora* or *Oidium*. Watery rot (also known as Oospora rot) is a common decay of tomatoes. It is caused by the fungus *Oospora lactis f. parasitica.* (Fig. 18) The mold grows best and produces most rapid decay at a temperature of about 85° F. It grows on cracked, broken tomatoes and is frequently found in the canning plant. This mold occurs on plant equipment if it is not kept scrupulously clean. It has a white, slimy appearance. Growing under these conditions, it commonly takes on a feathery appearance. (Fig. 25)

When this mold is observed in comminuted tomato products, it is good evidence of insanitary conditions in the plant equipment employed for packing the product. Equipment infected with this mold growth gives off a peculiar, characteristic fetid odor that can be readily detected.

Oospora (Oidium) mold propagates by the separation of cells from any part of the mycelium to form individual "oidia" or spores. The spores are 1½ to 2 times as long as they are wide and are essentially cylindrical with the ends somewhat convex. The filaments are septate. (Fig. 26)

Mucor and Rhizopus Rot

These two genera are grouped together since their appearance, manner of growth, and effect on tomatoes are quite similar. They are the types that produce the "leakers." Tomatoes which have been attacked by these molds become soft and mushy. There is no discoloration and the soft consistency of the rot gives it a water-soaked appearance as seen through the distended red skin. The rot develops deeply and rapidly and soon affects the entire tomato. The coarse mycelium of *Rhizopus* can be seen by carefully pulling apart the decayed tissue. This type of mold is prevalent in tomatoes that have been held for some time after picking before being canned. (Fig. 19)

The mold consists of coarse, whitish threads. It grows into the tissue and also aerially. The aerial portion has a loose, cottony appearance, and the spores are produced in little spherical shaped sporangia, which to the naked eye look like black specks scattered over the cottony mycelium. The spores are generally black or brown and the mycelium white or gray. The mycelium is coarse in texture, nonseptate, and has coarse, granular contents. (Fig. 27 and 28)

Fusarium Rot

Fusarium rot occurs in tomatoes from all producing areas in the country. It is most destructive on ripe tomatoes in the field. The symptoms of fusarium rot on vine-ripened fruits are a slight water soaking, sinking, softening and wrinkling of affected tissues. The decay progresses rapidly and may cause complete destruction in a few days. Any one of a number of different species of Fusarium can cause the decay. Entrance to the fruit is through wounds, insect injuries and lesions caused by other diseases. The causal fungi cause maximum decay at 75° F.

Fusarium mold appears early in the center of the spot of rot as a slightly raised, whitish or pinkish mass of mold which is quite characteristic to one who has become familiar with it. The spores are confined to microconidia unless sporodochia are formed, from which are issued the sickle-shaped spores. These spores may divide transversely by septae into two or more divisions. The mycelium is septate. (Fig. 29 and 30)

Penicillium Rot

Penicillium is a member of the so-called blue-green molds so often found on moldy bread. It is at times found upon tomato material, although it is

not as prevalent as some of the forms already mentioned. It will develop both in the field and in the plant. Penicillium mold is characterized by brush-like fruiting hyphae with numerous spores produced in chains at the ends of the finger-like extensions. The mycelium is septate. (Fig. 31) As a matter of interest, the antibiotic, Penicillin, is produced by the mold *Penicillium notatum.* Some cheeses are ripened by the use of molds belonging to the genus *Penicillium.*

Aspergillus Rot

Aspergillus is not common on tomatoes although it is occasionally found. Growth may occur in cracks and injured areas of the tomato, both in the field and in lug boxes. The hyphae are branched and septate. The hyphae enlarge at the apex of the conidiophores which bear the spores. The conidiophores are unbranched and usually long. This type of mold should not cause much concern in the micro-analytical examination of comminuted tomato products. (Fig. 32)

Botrytis Rot

Gray mold rot is a fungus decay caused by *Botrytis cinerea* auct. commonly found affecting market tomatoes but seldom in sufficient amount to make it of major commercial importance to the canner. The mold can enter the tomato through the unbroken skin or through cracks and injured areas. It may be classed as a field mold as it attacks tomatoes in the field. As indicated by the name of the rot, the mold grows over the decayed areas in the form of a prominent gray mold. (Fig. 33)

Phytophthora Infestans Rot

The fungus *Phytophthora infestans* is the cause of "late blight" rot on tomatoes. The amount of damage caused is directly related to the weather conditions during and a few weeks before harvest. Most serious losses occur during wet seasons when the nights are cool (50°-60° F.) and the days only moderately warm (60°-70° F.). Mean daily temperatures above 75° F. for one to two weeks check the disease. The spores of the fungus are killed within a few days during dry weather when the temperature reaches 80° F. or above.

Late blight often starts at the stem scar, but it can occur anywhere on the tomato.

Stemphylium Rot

Stemphylium or gray leaf spot, caused by *Stemphylium solani,* is a fungus disease of the foliage and occasionally affects the fruit. The disease is most severe in warm, humid weather and at times it causes a severe defoliation of the plants in both the seed bed and the field. (Fig. 35)

Cladosporium Rot

Cladosporium rot of tomatoes is caused by *Cladosporium herbarum.* Cladosporium rot may be of considerable importance in the field under conditions of high humidity and prolonged bearing of old vines. The fungus is most destructive when the temperature ranges between 65° to 80° F. (Fig. 36)

FIGURE 21
Alternaria—
Spores (X150)

FIGURE 22
Alternaria—
Spores and Mycelia
(X200)

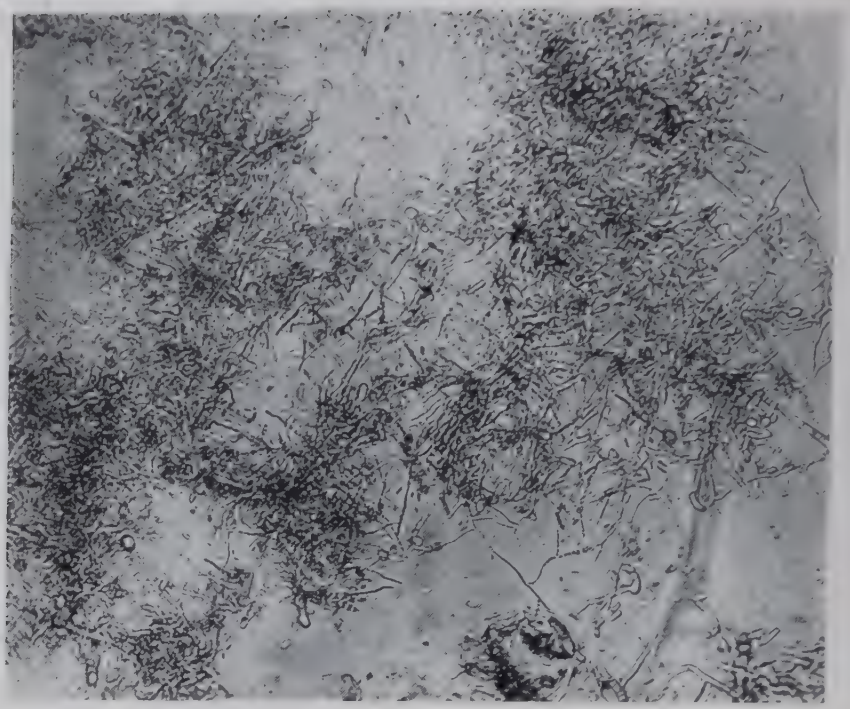


FIGURE 23
Colletotrichum (Anthracnose) on Tomato—Mycelia and Spores (X150)

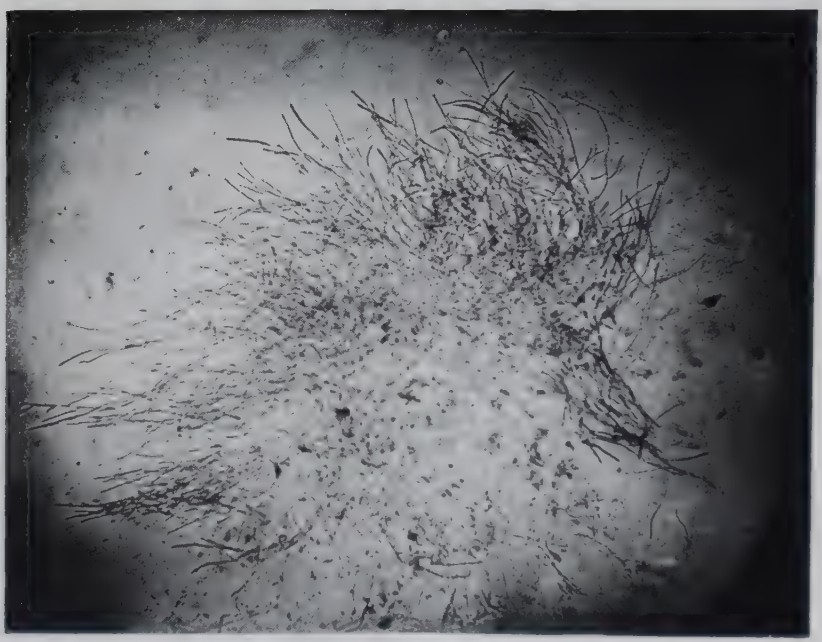
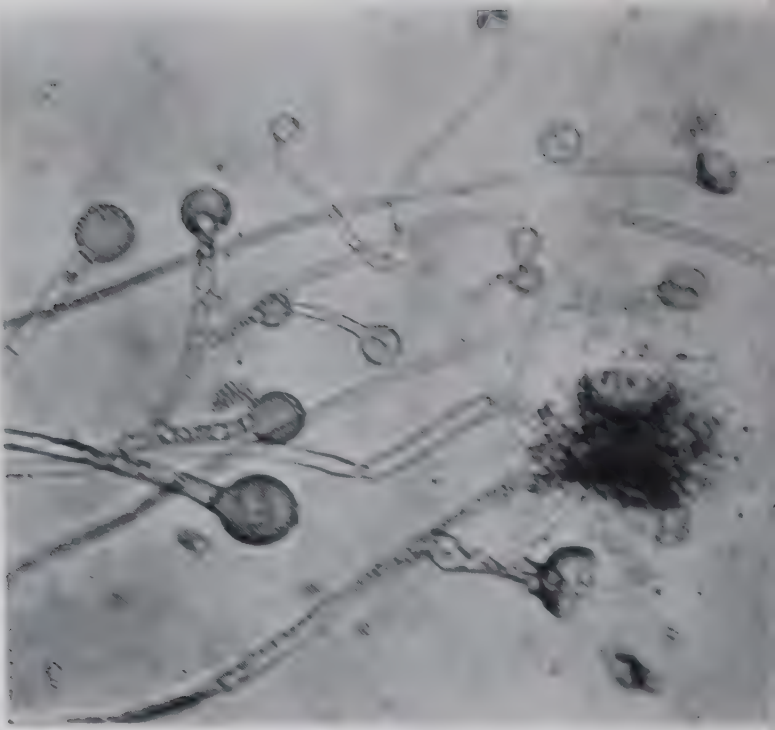


FIGURE 24
Colletotrichum phomoides—Anthracnose rot fragment from tomato puree (X75)

FIGURE 25
Oospora—
Machinery Mold
(X100)

FIGURE 26
Oospora—
Mycelia and Spores
(X150)

FIGURE 27
Mucor—Sporangia and Mycelia [X200]



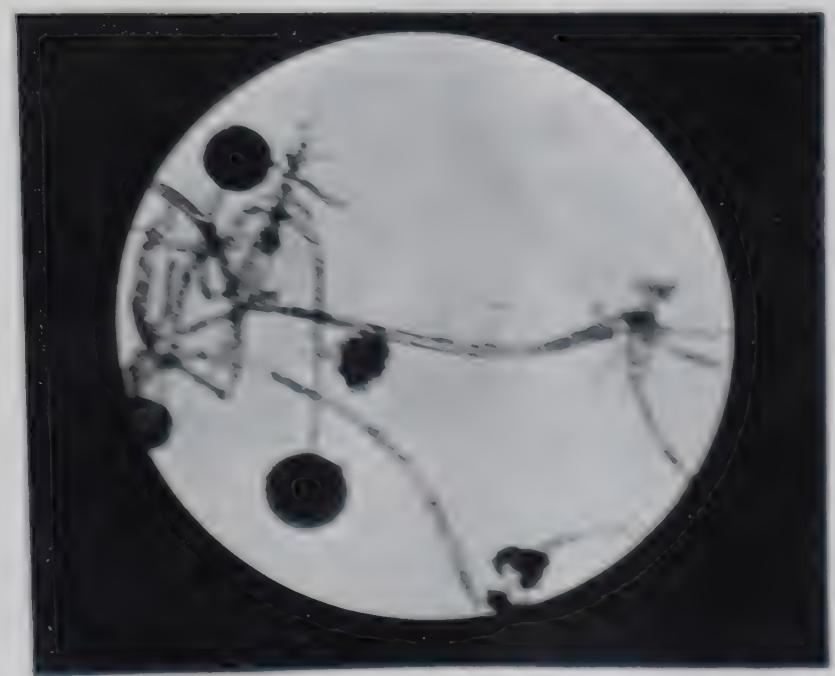
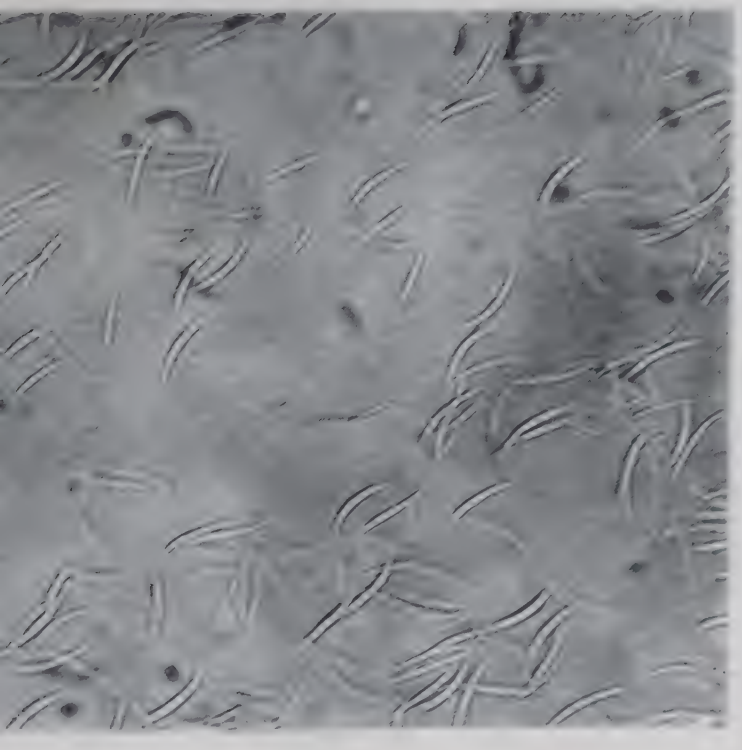


FIGURE 28
Rhizopus—Sporangial Head and Mycelia [X]



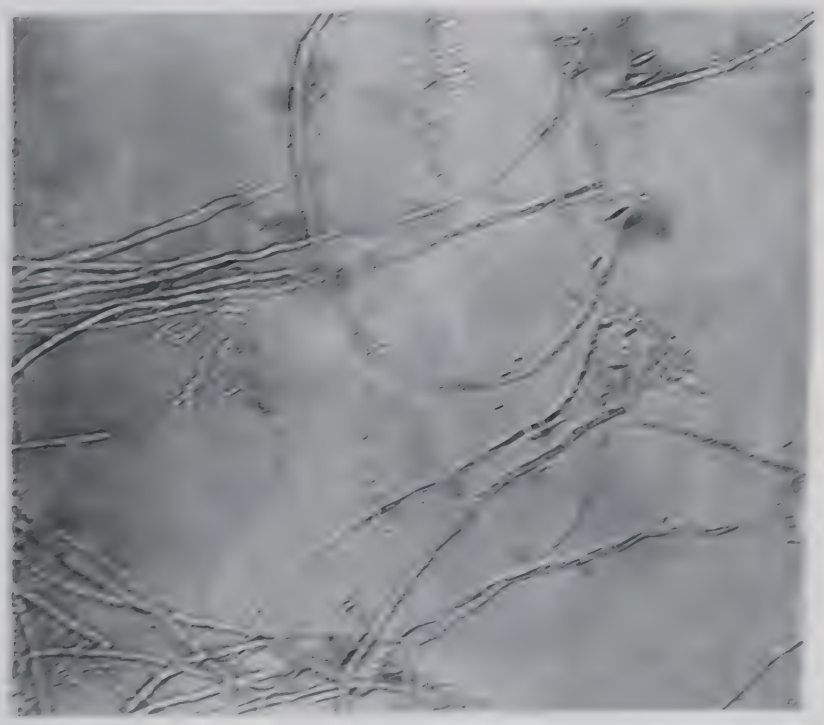


FIGURE 31
Penicillium—
Mycelia and
brush-like
conidial heads
(X200)

FIGURE 32
Aspergillus—
Mycelia and
conidial heads
(X100)

FIGURE 33
Botrytis—Mycelia and Spores (X100)

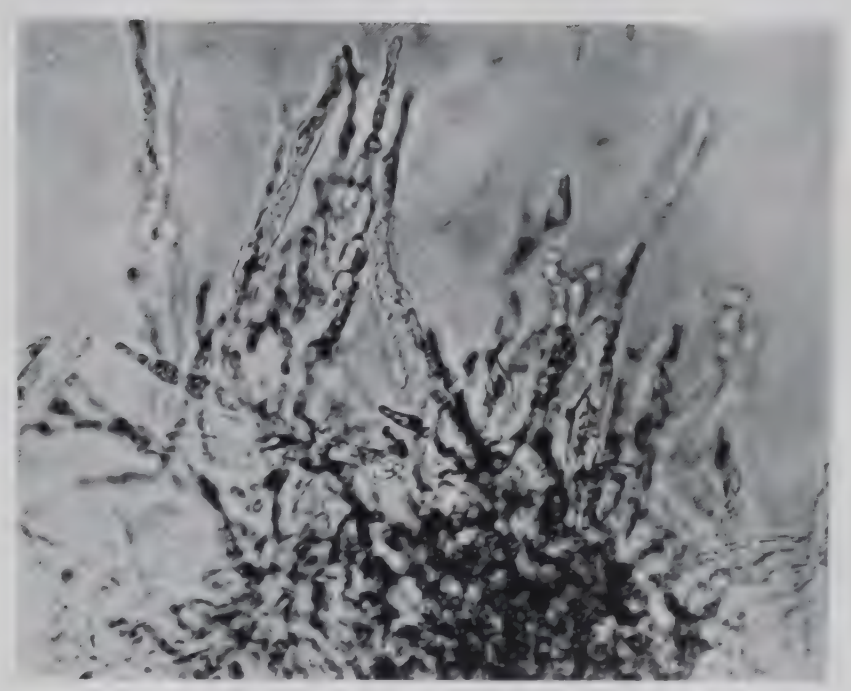


FIGURE 34
Phytophthora infestans Mycelia from a Blighted Tomato (X300)

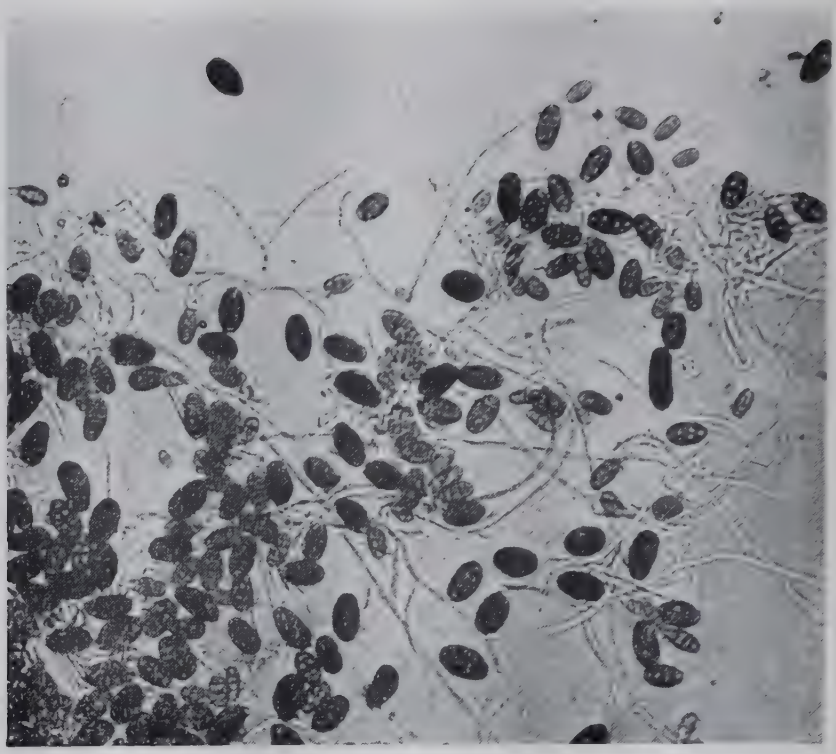


FIGURE 35
Stemphylium—
Spores and Mycelia
(X200)

FIGURE 36
Cladosporium—
Spores and Mycelia
(X200)

VIII. SOME FACTORS IN THE PACKING OPERATIONS WHICH AFFECT MOLD COUNTS

The control of rot in canned tomato products is the direct responsibility of plant management in the supervision of the packing operations. A high mold count is a post-mortem observation of conditions in the plant contributing rot.

Mold in tomato products may come from two sources: (1) moldy tomatoes as the result of poor sorting and trimming operations, and (2) contaminated plant equipment. Smith (10) discusses this subject in his article "Mold Count on Tomato Products, Part II. The Occurrence and Distribution of Mold Filaments in Tomato Products." Much of the following material pertaining to the sorting and trimming of tomatoes is based on information contained in this article.

Efficient Sorting and Trimming of the Tomatoes

Canners should use as good a raw product as possible so that a minimum of sorting and trimming is necessary. They should especially be on the lookout for any evidence of anthracnose infestation in the fields or in the loads of tomatoes received at the cannery.

Efficient sorting and trimming of the tomatoes require that each tomato be inspected on all sides and visible rot cut out by hand. Roller conveyers which turn the tomatoes continuously as they pass in front of the inspectors are particularly well adapted for this purpose. It cannot be emphasized too strongly that sorting and trimming of the tomatoes are separate operations. Those who are to sort out defective tomatoes must be given special training in this work. Those who do the trimming must also be given special instructions. Each group of sorters must be actively on the job whenever tomatoes are passing over the belt. All of the sorters must be shown the particular kinds of defects that must be removed from the tomatoes. They should be able to recognize anthracnose spots on tomatoes and to understand the difference between a crack that is healed over without growth of mold and one where there is evidence of mold development. The significance of sunburn or other affected portions of the surface which do not contain mold must be explained. Blight spots on tomatoes must be pointed out to the sorters. Tomatoes may be sorted both before and after washing, but the final sorting should be after washing. If the tomatoes are exceptionally bad with respect to mold, it is advisable to sort out and discard the badly decayed tomatoes before they go to the washer by use of a "dry sorting belt".

It should be emphasized that the sorting and trimming of the tomatoes is the most important operation with respect to insuring that they be free from mold. Inspection at the end of the belt where the tomatoes fall into the chopper or extractor, readily discloses whether the sorters and trimmers are adequately removing the rot. Those who trim the tomatoes which have been sorted out must be taught which

portions are to be cut out. Emphasis here is again on the importance of small spots of anthracnose. A relatively small amount of anthracnose may result in a high mold count. Every tomato that is set aside for trimming must be picked up and examined by the trimmer. Only the sound portions of tomatoes are used. In most cases, the rot is localized so that it is possible to cut out all of the rot and leave the remainder suitable for use. If there is any appreciable amount of anthracnose, however, this is very difficult. Usually, it is not worthwhile to trim tomatoes containing anthracnose; the entire tomato should be discarded.

Decayed portions of the tomato should be cut out using a sharp knife and not "dug out" with the fingers. Under *no* circumstances should the tomatoes be trimmed where the trimmings might fall on the sorting and trimming belt. The trimmers should be located at a separate table and the tomatoes trimmed directly over a water flume adjacent to the table or belt which conveys the trimmings from the factory.

Proper Lighting

Sorting belts should be well lighted. The best possible illumination is a good investment. Factories vary in this respect. According to a recent survey on lighting, the optimum level of illumination for tomato sorting and trimming operations should range between 80 and 100 foot candles. (15) Lights are preferably placed below the level of the eyes of the inspectors, with proper shields so that the light goes on the table and not in the eyes of the workers. The lights should be diffused rather than glaring.

Speed of the Sorting Belt

The speed of the sorting belt must not be substantially greater than 25 feet per minute. The tomatoes passing the inspectors must not be more than one layer deep.

Efficient Washing of the Tomatoes

Tomatoes, like most field grown crops when delivered to the cannery, have some dirt and other foreign matter, including mold, on the surface of the fruit. During or following a period of wet weather, this is substantially increased and usually a considerable amount of mud adheres to the tomatoes. Therefore, it is important that the tomatoes be efficiently washed. The washing of tomatoes prior to processing has been largely a matter of the canner's personal opinion and experiences. An industry survey indicates that a soaking period followed by spray washing, or spray washing alone, is used in most instances. The spray washing methods employed vary from water dripping from holes drilled in pipes to patented nozzles operating under relatively high water pressure.

The following suggestions are offered as a guide for efficient washing operations:

1. Water pressures between 80 and 100 pounds per square inch as indicated by a gauge placed in the water line just back of the spray outlets, are advocated depending upon nozzle design.

2. Whether the washing is done by a rotary cylinder or a roller belt, the tomatoes should not be more than one layer deep.

3. A combination of flat and solid cone spray-type nozzles is believed to give the most effective washing action. Use a spray which produces coarse drops, not a fog spray. The base of the water cone should intersect with the adjacent cones not more than one inch above the level of the top of the tomato layer. The outlet end of the nozzle should be not more than ten inches from the top surface of the tomatoes. (Obviously, all sprays should function properly and be checked regularly to see that none is clogged.)

4. The tomatoes should be under continuous spray for not less than 3 minutes and should be rotating constantly while under the spray at a rate of not less than 6 to 10 r.p.m. For unusually soft tomatoes this time should be doubled.

5. In areas where the tomatoes are grown on fine clay or sandy soils, the tomatoes should first be soaked in a running water bath (such as a flume) for not less than 4 to 6 minutes in order that the dirt may be removed prior to pressure-spray washing. If a soak tank is used, the water in the tank should be drained at least twice a day and the tanks cleaned and refilled with fresh water. A false bottom soak tank with suitable outlets has been suggested to permit frequent removal of the accumulated soil.

Two general difficulties encountered in constructing spray washing systems are: (1) a failure to provide sufficient water to operate the system, and (2) failure to use sufficiently large inlet pipes to carry the volume of water required and compensate for pressure loss in the system. It is suggested that, prior to the installation of a spraying system, the manufacturers of such equipment be contacted to obtain the benefit of their experience in the design and layout for a specific plant to insure maximum efficiency with economy of operation.

It is not suggested or implied that the washing operations should replace the sorting and trimming of the tomatoes but to supplement them. The mold filaments penetrate the flesh of the tomato, and trimming is necessary to remove the mold. Many packers have found it desirable, as the tomatoes leave the pressure spray washer, to have them pass under another short set of pressure sprays as they enter the sorting belt and again after sorting, just prior to entering the chopper or extractor. In order to avoid the possibility of added water being carried over into the chopper or juice extractor, it is suggested that the latter set of pressure sprays be located approximately 10 feet from the end of the sorting belt and that this portion of the conveyor unit be either a metal roller type or of open metal wire construction to permit proper drainage.

Washing of Sorting Belts

Sorting belts should be kept clean and free of extraneous refuse. A set

of sprays, using chlorinated water, located at each end on the underside of the sorting belt as it makes its return trip is very helpful for this purpose.

The sorting and trimming belts should be completely emptied of tomatoes and the belts thoroughly cleaned prior to any extended shut-down period, such as lunch hour, etc.

Clean Plant Equipment

There is no excuse for the accumulation of mold growth on factory equipment. All surfaces exposed to the tomatoes or the juices must be kept free from tomato refuse and visible dirt. The inspection table and belt should be constructed so that every part can be seen and cleaned. The under surfaces of the belt and guard rails are often neglected. A strong stream of cold chlorinated water is usually sufficient for cleaning, but if not, this should be supplemented by brushing or high pressure jets. Juice lines should be examined for dead ends. An article by Wildman and Clark (16) describes some examples of machinery slime in tomato factories. They found mold accumulation on certain equipment, especially the sorting tables and were able to identify the typical "machinery mold" in the final product.

Too much emphasis cannot be placed upon good plant sanitation, especially with respect to the cleaning of wood pulp tanks, pipe lines, finishers and shaker screens. A number of instances have been observed where the sorting and trimming operations were good and the juice from the extractor had a low mold count but the mold count of the finished product was high. Obviously, this indicates contaminated equipment as the source of the trouble.

Sanitation control in the tomato factory, as in all food processing plants, is essential for the production of high purity products. Briefly, sanitation means keeping the plant and equipment clean. To do this requires the establishment of a definite sanitation program. The primary responsibility rests entirely upon plant management. Top management should familiarize themselves with the problems of sanitation in their own plant. If possible, some one individual should be appointed "plant sanitarian" and serve as a full-time employee. If a part-time sanitarian is appointed, then he must be relieved of sufficient of his other duties to give him freedom to spend sufficient time on sanitation. The plant sanitarian should be responsible directly to management. He must function in both regulatory and advisory capacities to insure that the tomato products produced or handled meet all the sanitary requirements. An efficient, continuous sanitation program should be so planned and organized as to maintain at all times a good appearing plant, producing high quality products.

IX. EFFECT OF HOMOGENIZATION ON MOLD COUNT

Homogenization is employed by some packers in their operations for packing tomato juice. The effect of homogenization on the mold count is irregular due to the character of the mold. However, the mold count is

TABLE II.

TOMATO JUICE—EFFECT OF HOMOGENIZATION ON MOLD COUNT

Percent by Weight Visible Rot to Extractor	Mold Count Percent Positive Fields	
	Before Homogenization	After Homogenization
0.13	10	10
0	0	8
Trace	12	16
0.16	8	6
0	0	26
0	4	31
0.52	4	24
0.05	6	34
0.44	16	26
0.28	18	20
0.14	18	28
0.06	10	12
0.02	10	30
0.12	10	20
Trace	10	10
0.26	16	33
0.10	6	14
0.04	10	24
Average 0.13	9.3	20.6

usually higher after homogenization as the pressue employed breaks up the clumps of mold that are present into separate filaments, and the individual filaments are sometimes broken into smaller pieces. Smith (4) in his paper "The Relation of Visible Rot to Mold Counts", shows the effect of homogenization on mold count of tomato juice, as expressed in Table II.

"The above data are in line with general knowledge previously obtained but show numerically the extent of such increase in the mold count."(4)

The Federal Food and Drug Administration recognizes the possible effect of homogenization on the mold count as published in their official statement released on July 17, 1940:

> ". . . Adequate allowances will be made in applying the tomato juice tolerance to those articles which have been subjected to a homogenization process."

X. RECENT ANNOUNCEMENT OF U. S. FOOD AND DRUG ADMINISTRATION ON COMMINUTED TOMATO PRODUCTS

The Food and Drug Administration recently clarified their attitude with regard to the presence of mold and rot in comminuted tomato products. On July 20, 1951, they issued a *Trade Press Memorandum* (17) about comminuted tomato products which was sent to canning industry trade papers to furnish them with background information. On the same date, they issued the "Statements of General Policy or Interpretation, Comminuted

Tomato Products Containing Rotten Tomato Material" which was published in the Federal Register, July 20, 1951, and in the N.C.A. Information Letter of July 21, 1951.(18)

In view of the importance of this information to canners of tomato products, these reports are included here in their entirety.

Trade Press Memorandum— July 20, 1951

"Factory inspections have led to the conclusion that many manufacturers of tomato products (catsup, chili sauce, paste, etc.) have been placing too much emphasis on mold count as an indicator of the condition and suitability of the raw stock for packing. No doubt Food and Drug Administration's well known policy of initiating legal actions on the basis of mold count has had a great deal to do with this. It has become evident, however, that abuses have arisen. Some firms are relying on mold count rather than emphasizing their sorting and trimming operations.

"Some firms have their own mold counting laboratories and make frequent checks during daily production. When the mold count is running safely low, it is a common practice of some packers to cut down on the sorting and trimming operations and increase the loading of the sorting belts, event though substantial portions of rotten tomatoes may be going into a product as shown by visual inspection. Some firms even have green-amber-red signal light systems, operated from the mold count laboratory and visible to the supervisor of sorting and trimming. This directs the addition or substraction of personnel from those operations, depending on the mold count, sometimes without any regard to the visual appearance of the tomatoes on the inspection belt.

"Other smaller firms regularly send samples to commercial laboratories for counting. Results on these samples may determine whether the production will be withheld from interstate commerce, blended with other products to produce a low mold count, or used in the manufacture of other foods where the mold count will be reduced or at least partially obscured.

"Rots caused by bacteria and viruses do not increase the mold count of tomato products. Further, even the several types of fungus rots that are characterized by mold filaments vary considerably in the extent to which the mold count will be increased. During the 1950 packing season, tomatoes in some areas were affected by a soft rot which was apparently caused largely by bacteria, virus, or a type of fungus characterized by small numbers of mold filaments. Some packers used from 30 to 50 per cent of tomatoes affected with this type of rot without materially raising the mold count, and regarded their product as legal.

"The attached notice is aimed at correcting the situation by emphasizing the need for adequate sorting and trimming, and that products made from unfit material cannot be rendered legal by blending with other material.

"It should not be concluded from the foregoing that mold count is no longer of importance. High mold count is evidence of decomposition,

but mold count should not be used as a substitute for inspection, sorting and trimming. Packers who are careful of the quality of raw materials used and emphasize adequate sorting and trimming in addition to the mold count will not be affected by this notice." (17)

Statements of General Policy or Interpretation: Comminuted Tomato Products Containing Rotten Tomato Material

"Pursuant to section 3 of the Administrative Procedure Act (60 Stat. 237, 238; 5 U.S.C. 1002), the following statement of policy is issued:

"S 3.24 notice to packers of comminuted tomato products. It has long been known that tomato rot may be caused by one or more of the following: fungus diseases, bacterial diseases, virus diseases, and certain nonparasitic diseases. Only the fungus rots are characterized by the presence of mold filaments. Mold counts on comminuted tomato products are not increased by incorporating within the product tomato rot caused by bacteria, virus, or nonparasitic factors. Although high mold counts on these products reveal that large amounts of rotten material are present, low mold counts do not necessarily demonstrate absence of the type of rot caused by the tomato diseases that are not characterized by mold filament.

"Inspections of canneries engaged in the packing of comminuted tomato products show that most packers effectively trim, sort out, and discard rotten tomatoes from the raw stock. Some packers, however, do not properly eliminate rotten tomato material, and a few packers deliberately use rotten tomatoes in these foods, provided the mold count remains low. Some packers, on occasion, have mixed tomato products having a high mold count with tomato products containing little or no mold, so as to produce a blend with a low mold count.

"Packers of comminuted tomato products who rely upon the mold count as the sole or primary control procedure, to the neglect of adequate sorting and trimming, may produce products with low mold counts which contain substantial amounts of rot.

"It is the purpose of this announcement to advise all canners of tomato products that:

"(a) Although high mold count is conclusive evidence of inclusion of substantial amounts of rot, mold count is not the only way of establishing that comminuted tomato products contain decomposed tomato material.

"(b) Where factory observations or other evidence reveals that comminuted tomato products contain rot not caused by mold, such rot, as well as that caused by mold, will be taken into account in applying the provisions of the Federal Food, Drug, and Cosmetic Act against adulteration.

"(c) The blending of tomato products adulterated with tomato rot, of whatever kind, with tomato products made from sound toma-

toes, or with other sound food, renders the blend adulterated.

(Sec. 701, 52 Stat. 1055; 21 U.S.C. 371)

Dated: July 16, 1951

John L. Thurston
Acting Administrator

(F.R. Doc. 51-8343; Filed, July 19, 1951; 8:55 A.M.)" (18)

The foregoing statement of policy clearly indicates the attitude of the Food and Drug Administration relative to evaluating the purity of comminuted tomato products. They still regard high mold counts as indicative of decomposition. On the other hand, low mold counts do not necessarily indicate sound stock and are not conclusive evidence that proper care has been used in the removal of all types of objectionable rot. Rots which are caused by bacteria, viruses and physiological causes are unaccompanied by mold and are not detected by this method. According to Eisenberg, (3) "the Administration also relies on factory inspections as a means of enforcing the Federal Food, Drug and Cosmetic Act. The inspector takes scrapings of any slimy tomato accumulations and makes a thorough examination of equipment to judge proper cleaning. He examines the sorted and trimmed stock to judge the effectiveness of this operation in eliminating rotten tomatoes. Laboratory and inspectional techinques are used by the Administration to supplement each other and give a complete picture of a factory's operations, thus making it possible to judge each case on its merits." The Administration also considers it illegal for comminuted tomato products containing decomposed material to be used as ingredients of manufactured foods such as tomato soup, spaghetti sauce, etc., since this would constitute adulteration of the fabricated food and render the latter subject to action.

The Howard Mold Count will continue to be used as one of the official methods for the examination of comminuted tomato products to detect the presence of decomposed tomato material. It is therefore, essential that packers of tomato products have a thorough understanding of the method and its application.

XI. MOLD COUNT TOLERANCES

Notices of Judgment under the Federal Food and Drug Act, June 30, 1906, show that regulatory actions were instituted using the principles of the Howard Mold Count Method as early as 1913. Each year since that time a substantial number of the Notices of Judgment have been based on this test.

Federal Food and Drug Administration tolerances based on the Howard Method have been established for tomato products. Federal tolerances apply to interstate shipment and state tolerances apply to products packed and sold within the state. Most states have standards that conform to the federal tolerances.

The first Government mold count tolerance was established in 1916. This tolerance together with the changes which have been made subsequently are shown below:

FEDERAL MOLD COUNT TOLERANCES FOR TOMATO PRODUCTS

Date	Percent Positive Fields	
	Tomato Juice	Other Comminuted Tomato Products Catsup, Puree, Paste
September 5, 1916	—	66
May 14, 1931	—	50
July 1, 1936	35	50
July 27, 1938	25	50
June 17, 1940	15	40
May 13, 1941	20	40

On May 13, 1941 the Food and Drug Administration announced that the tolerance on tomato juice would be set up at 20 per cent instead of 15 per cent. In making this change, Mr. W. G. Campbell, then Commissioner of Food and Drugs, released the following statement:

"On June 17, 1940, the Administration announced reductions in previously established mold count tolerances for tomato juice, catsup, puree and paste. Subsequently, industry appeals from these reductions were received with representations that in spite of the utmost care in properly conducted plants, occasional adverse conditions are reflected in mold counts somewhat exceeding the new tolerance.

"The Administration thereupon undertook an exhaustive appraisal of the situation. The facts developed justify the conclusion that in the case of tomato catsup, puree and paste it is not unreasonable to expect canners to meet the mold tolerance of 40 per cent of the microscopic fields announced in 1940 and actions will continue against shipments when this figure is exceeded.

"In case of tomato juice, the investigation indicated that under abnormal conditions beyond the control of the packer, mold counts may exceed slightly the tolerance of 15 per cent of the fields announced for tomato juice. In recognition of this possibility, the Administration will not institute actions against tomato juice on the ground of excessive mold count unless mold filaments are present in more than 20 per cent of the microscopic fields. Adequate allowances will be made in applying the tomato juice tolerance to those articles which have been subjected to a homogenization process. Since the Administration is convinced that with more experience the industry can universally meet more restricted tolerances, the present announcement is subject to the qualification that it is set pending consideration of further reductions.

"Comminuted tomated products exceeding these mold count tolerances

cannot be used as ingredients of manufactured foods such as tomato soup, spaghetti sauce, etc., since this would constitute adulteration of the fabricated food and render the latter subject to action."

No government mold count tolerance has been established for canned tomatoes. However, when juice or puree are used as packing media, the respective mold count tolerances for these products apply, and it is the packer's responsibility to control and check his packing medium prior to its mixture with the whole tomato stock.

During the 1953 canning season, the National Canners Association, (19) investigated the problem of mold count on the drained juice of canned tomatoes. The results of these tests show that when tomato juice is added to canned tomatoes, the liquid drained from the canned tomatoes has a mold count less than that of the juice that was added. The extent of the reduction in mold count varies according to a number of conditions. When the amount of liquid drained from the canned tomatoes is much greater than the amount of juice that was added in packing, the reduction in the mold count is quite large. However, when tomato juice is added as a packing medium for fancy whole tomatoes and the amount of liquid drained from the canned tomatoes is only slightly greater than the amount of juice added, the mold count of the drained liquid is only slightly less than that of the juice used. In general, the reduction in the mold count is greater than can be accounted for by dilution only. In draining the liquid from canned tomatoes by the usual drained weight procedure, a considerable part of the fine solids in the liquid portion stays on the screen with the tomatoes. A corresponding proportion of the mold present seems to remain on the screen with the fine solids so that the liquid drained off has less mold than would otherwise be found. The studies show that the mold counts vary greatly with individual cans in the same lot, but the mold count pattern of the packing medium can be determined from the analysis of a sufficient number of cans for mold count and the consistency of the drained tomatoes. The complexity of this problem does not lend itself to the simplicity of a single figure as a dilution factor for all types of canned tomatoes.

Canadian Tolerances

The Meat and Canned Foods Act, R.S., c 77, S.1. and Processed Fruit and Vegetable Regulations bulletin published by the Minister of Agriculture, Ottawa, Canada, 1954, establishes the following mold count tolerances for tomato products:

> "(a) (i) Tomato Juice, Tomato Juice Cocktail, when examined according to the "Howard" Method, mould filaments shall not appear in more than 25 per cent of the microscopic fields; . . ."

> "(ii) Tomato catsup, paste, puree, pulp, soup, tomato sauces, when examined according to the "Howard" Method, mould filaments shall not appear in more than 50 per cent of the microscopic fields; . . ."

XII. RESPONSIBILITY OF PLANT MANAGEMENT AND THE ANALYST

Every canner wants to pack a clean wholesome product. However, the mold count at times may indicate the presence of excessive decomposed tomato material in the finished product. When a high mold count is reported by the analyst, the organizational set-up in the plant should be such that this information can be channeled to the proper person or persons for immediate action. In order to use the information supplied by the analyst to best advantage, it is necessary that plant management — the Superintendent or Supervisor of Quality Control — be familiar with the factors that cause high mold counts and with corrective measures necessary to insure the packing of a clean wholesome product. Once the analyst has reported his findings to the officially designated supervisory personnel, his responsibility should end. Plant management should then be able to locate and correct the source of trouble immediately. This may require special training of the superintendent, foreman, forelady or other supervisory personnel so they will know the corrective measures to take to reduce the mold count. Occasionally, it may be necessary to have the analyst assist by taking a set of "line samples" from each significant unit operation: e.g. chopper, extractor, cook tank, finisher, supply tank, filler bowl and can of juice immediately after closing, in order to locate sources of trouble resulting from contaminated equipment.

In order to maintain the most efficient canning operations in the manufacture of tomato products of high quality, it is essential that close cooperation between plant management and the analyst be maintained. This requires their mutual respect and good will. The analyst must be adequately trained and competent in using the Official Howard Mold Count method. Plant management must be able to interpret intelligently the results supplied by the analyst, know what corrective measures are necessary to insure a clean wholesome product and put these measures into effect immediately.

It is also the responsibility of management to supply the analyst with a good microscope and other necessary equipment, and to provide a suitable place for him to work. The table and the chair or stool should be of such heights that observations can be made by the analyst without straining of the neck or back. Before the start of the tomato canning season, the microscope should be inspected and cleaned to insure serviceability. Make sure an extra Howard mold slide, coverglasses and spare light bulbs for the microscope are available. A packer cannot afford to be interrupted during the pack because of failure to provide necessary equipment for efficient performance. An article by Troy (20) "Responsibility for Control of Mold Count of Tomato Products" gives additional suggestions on this subject.

Coding of Cans

One of the most important responsibilities of plant management is to make sure every can of finished product is coded and properly identified. The importance of coding cannot be overemphasized. This may be done by us-

ing an embossed code on the cans. Each can of tomato product should be coded to identify it by batch or by other subdivision of the pack. Where possible, the code should be changed for each batch; where continuous operation is used, it should be changed at least once a day, and preferably after short operating intervals during each day. Some packers change the code every two hours; others after extended shutdown periods; noon, evening, etc. Such identification enables a canner to separate and segregate specific small lots of a given product in case of seizure or controversy, and may thereby serve to reduce any loss to a small fraction of the entire pack. Use of no code or only a single code throughout an entire season is dangerous and may result in the loss of the entire pack if high mold counts have been encountered, as there is no way provided for making a satisfactory segregation.

XIII. APPENDIX HOWARD MOLD COUNT EQUIPMENT

A. Optical Apparatus

1—Monocular microscope with draw tube, without oculars, objectives, or other accessories. If a binocular microscope is desired, it should be purchased with the specification that it be calibrated to give a microscopic field of vision of 1.382 mm. in diameter (area 1.5 sq. mm.)

1—Abbe condenser with one iris diaphragm.

1—16 mm. achromatic objective.

1—8 mm. achromatic objective.

1—10X huygenian ocular with drop-in micrometer disc cross ruled in sixths of the ocular diaphragm opening.

1—Mechanical stage.

1—Substage lamp with daylight glass.

B. Other Equipment

1—Howard mold counting cell with calibration circle, or lines, for obtaining exact field size and two cover glasses.

1—Scalpel.

1—Dissecting needle.

1—Beaker (250 to 600 cc.).

1—Clean towel or lint free cloth suitable for drying mold counting cell and cover glasses.

THE MICROSCOPE

A Simple Microscope is an ordinary magnifying glass. The compound microscope differs from the simple microscope in that it has two separate lens systems. The one nearest the specimen, called the objective, magnifies the specimen a definite amount. The second lens system, the eyepiece, further magnifies the image formed by the objective so that the image seen by the eye has a magnification equal to the product of the magnifications of the two systems.

The image formed by a compound

microscope is inverted; the object is seen upside down and reversed so that the right side is at the left. Movement is reversed also, but one soon learns which way to move the slide.

Construction of the Microscope

The names of the various parts of the microscope are shown in Figures 37 and 38. The basic type of compound microscope, consists of the eyepiece, objective, and a tube which holds them at the proper separation. The instrument is focused by a rack and pinion. For convenience of manipulation, the microscope is mounted on a stand with a stage to support the specimen.

The function of the principal parts of the microscope are described below:

A. The Ocular

The function of the ocular or eyepiece is to magnify the image formed by the objective and to position the micrometer disc. The ocular and micrometer disc should be scrupulously clean and free from dust, lint or smear. By rotating, one can determine if visible particles are in the ocular or on the slide.

B. Adjustments

All good microscopes are equipped with two adjustments; coarse (quick acting) and fine (slow acting). The large knobs are used as a quick adjustment to focus the tube proper with respect to the stage that holds the mold count slide. The fine adjustment or small knobs must be used *constantly* in order to focus at various depths of the sample. For instance, it is possible for only half of a mold filament to be in focus in one position. By raising or lowering the tube with the fine adjustment the true dimension of the entire filament can then be observed. It should be remembered, in examining tomato products for mold count, the depth of sample as established by the special Howard Mold Count slide is 0.1 mm. deep. The optics of the microscope will not image the full depth in any one position; therefore, constant use of the fine adjustment is mandatory for accurate results.

C. Objectives

Microscopes are supplied with one or more objectives mounted on a nosepiece or turret. In mold counting, the 10X-16 mm. objective is used. This objective is calibrated with the 10X ocular to produce the desired microscopic field diameter of 1.382 mm. (1.5 sq. mm. area) as specified in the Official Howard Mold Count Method. In those instances where identifying characteristics of mold filaments are not clearly discernible, the official method suggests the use of 200X magnification (8 mm. objective) to confirm the identity of the mold filaments.

The function of the objective is to form an image which is intercepted and magnified by the ocular. Keep the lower surface of the objective absolutely clean. A slight film can materially impair the function of this, the most important element of the optical system. Xylol may be used sparingly to remove foreign matter and should be wiped clean with lens paper.

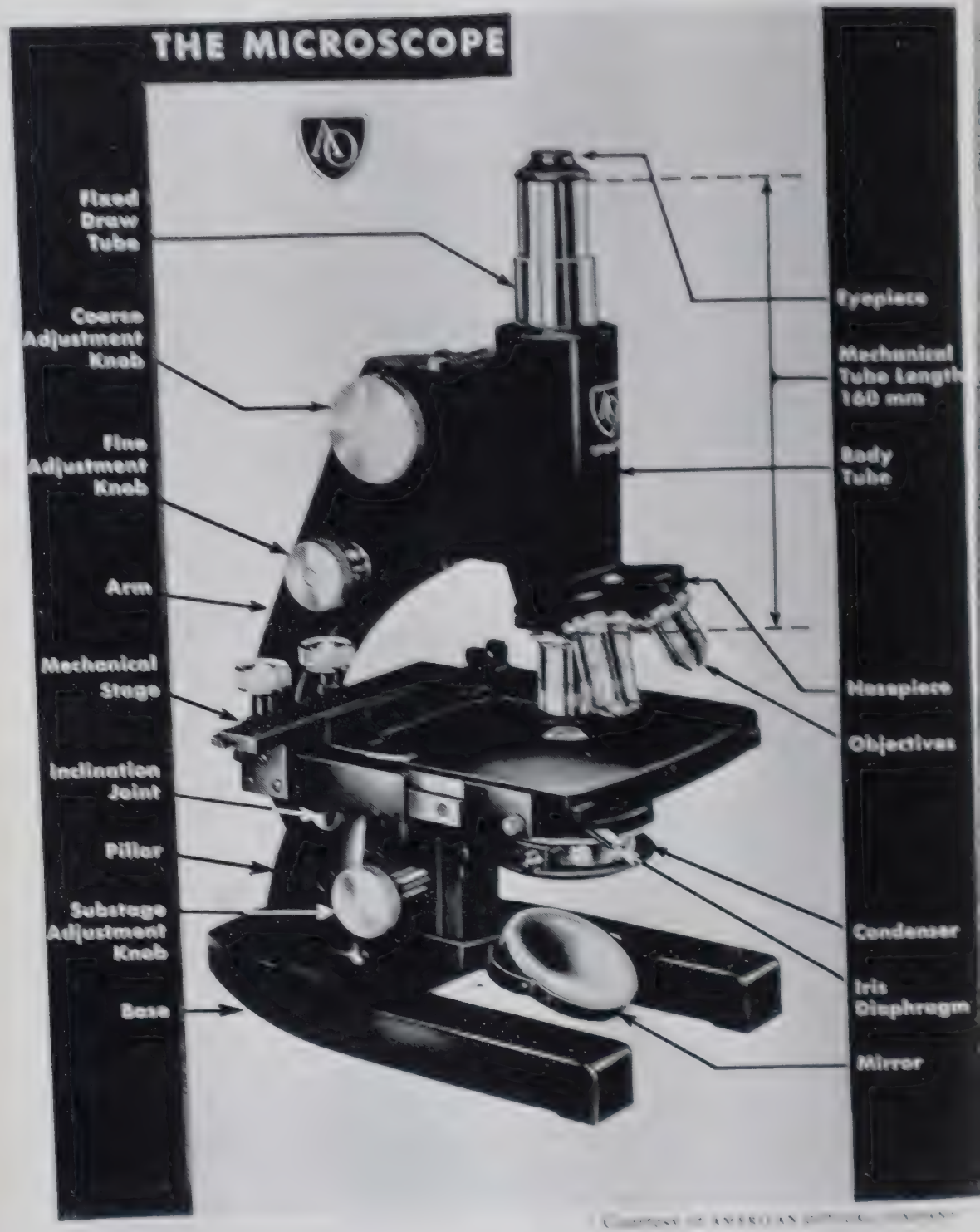


FIGURE 37

Monocular Microscope

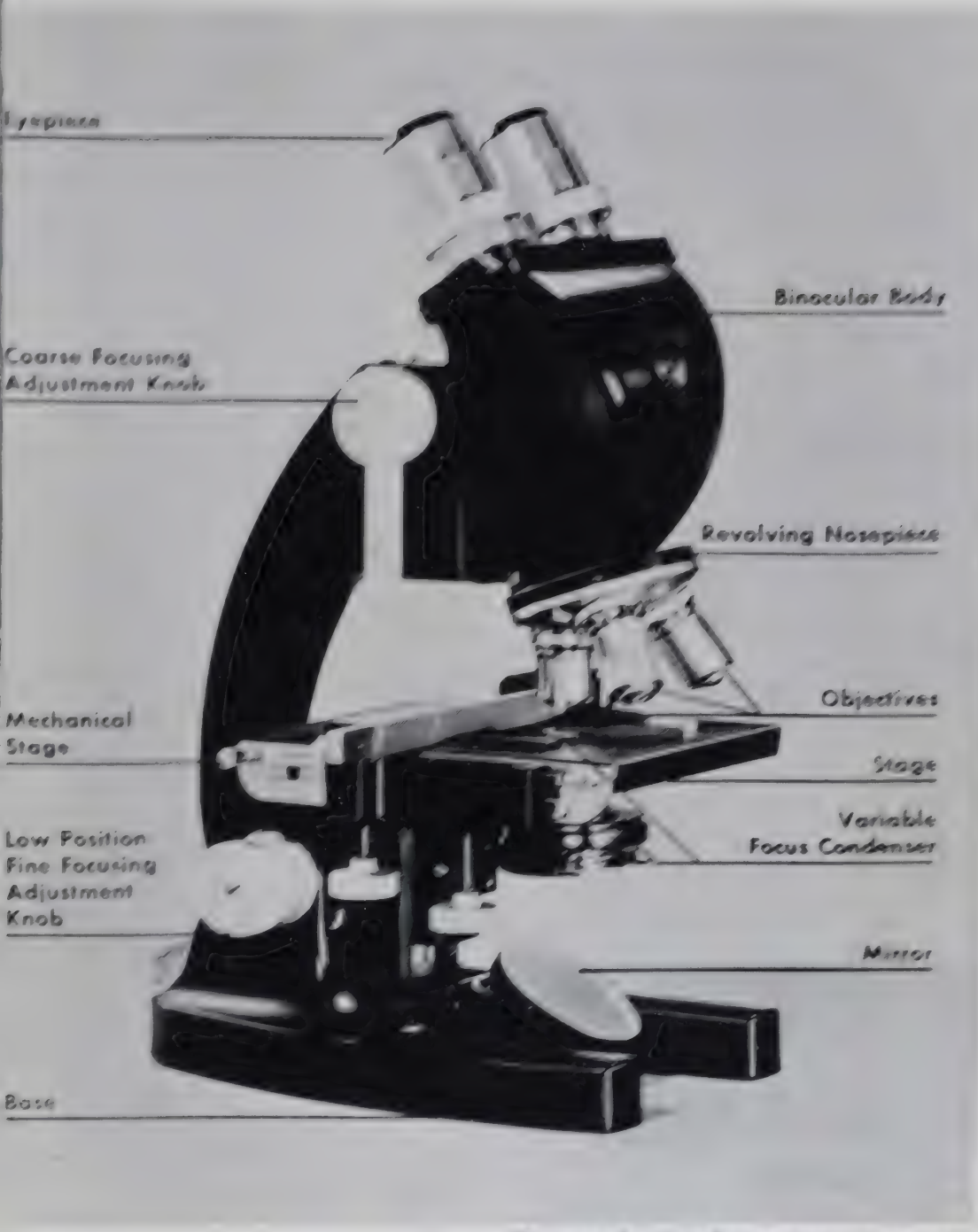


FIGURE 38

Binocular Microscope

D. Mechanical Stage

This required accessory enables systematic observation of the specimen. It is impossible to manually shift position of the mold count slide and attain any degree of accuracy. The mechanical stage provides to and fro and lateral movements. Good care is essential. Clean once or twice yearly by removing all lubricant from slideways with xylol. Wipe dry and relubricate sparingly with vaseline.

E. Condenser

The condenser located directly beneath the stage is of extreme importance. The correct use of the condenser in controlling the light will increase or decrease the visibility of microscopic particles. If the condenser is opened too far, fine details cannot be seen; fine molds may be overlooked as the result of too much light. If the condenser is closed too far, diffraction patterns reduce visibility. To obtain maximum clarity of image, gradual opening and closing of the iris diaphragm (the small lever usually located on the right side) will reveal the desired position for optimum performance. The function of this is similar to the diaphragm setting on a good camera lens. Raising or lowering of the condenser by means of the attached rack and pinion will also facilitate proper illumination.

Most microscopes are equipped with condensers that are divisible. For low power observations such as 100X magnification, used for mold counting, the top element of the condenser can be removed to achieve more uniform illumination.

F. Mirror

The mirror has two surf
concave and the other fla
(bottom of the condenser)
the flat side of the mirror
conversely, if the condens
vided, the concave side of
will produce the best illum
examining specimens
slight adjustment of the po
tion produces slightly ob
which results in better reso

G. Light

Proper illumination of
important if the most pe
tion of fine details of micro
jects is to be attained. In
nation of tomato produ
presence of mold filaments
light will conceal fine det
little light will not reve
distinctly. The light sh
justed properly for each
sample.

Monocular microscopes
light than binocular type
microscope lamps, 110 V
bulbs, for both the separa
tor and the illuminator at
condensers are adequate for
scopes. For optimum p
binocular microscopes,
watt built in a lamp with
and a corning filter.

Care of the Microscope

The microscope should
its arm and, when not in
be placed in a case or pro
to protect it from dust
must be kept scrupulously

References

1. Howard, B. J. *Tomato Ketchup under the Microscope with Practical Suggestion: to Insure a Cleanly Product.* U. S. Department of Agriculture, Bureau of Chemistry, Circular No. 68. February 13, 1911.

2. Statistics Division, National Canners Association, Washington, D. C. *Annual Production Report for Canned Tomatoes and Tomato Products.* 1955.

3. Eisenberg, W. V. *Observations and Suggestion on Factory Control of Rot and Extraneous Matter in Tomato Products.* National Canners Association Information Letter No. 137: 36. January 30, 1952.

4. Smith, Howard R. *The Relation of Visible Rot to Mold Counts.* National Canners Association Information Letter No. 137: 32. January 30, 1952.

5. Howard, B. J. and C. H. [illegible]. *Microscopical Studies on Tomato Products.* U. S. Department of Agriculture, Bulletin No. [illegible] (reprint) 1917.

6. Association of Official Agricultural Chemists. *Official Methods of Analysis of the Association of Official Agricultural Chemists.* 8th Ed. Washington, D. C., Association of Official Agricultural Chemists. 1955.

7. Bigelow, W. D., H. R. Smith, C. A. [illegible], L. W. Bowlby, and I. M. Reed. *Tomato Products.* National Canners Association Research Laboratory, Washington, D. C. Bulletin No. 27-L Revised July 1950.

8. BIGELOW, W. D. *Mold Count on Tomato Products.* The Canner 70:11, Part 2, pp. 50-55. February 23, 1933.

9. W[illegible], J. D. A Statistical Study of Some Sampling Relations with Special Reference to Quantitative Microscopy. Journal of Association of Official Agricultural Chemists. 14, No. 4: 565-570. 1931.

10. SMITH, HOWARD R. *Mold Count on Tomato Products.* Part I. [illegible] the Identification of Mold Filaments. The Canning Trade. 70: No. 36: 8-9. March 29, 1948. Part II. The Occurrence and Distribution of Mold Filaments in Tomato Products. The Canning Trade. 70: No. 50: 7-8, 20-21. July 5, 1948.

11. SMITH, HOWARD R. (Personal Communication) 1950.

12. The Microanalytical Division, Food and Drug Administration, Federal Security Agency. Microanalysis of Food and Drug Products. U. S. Government Printing Office. Washington, D. C., Food and Drug Circular No. 1. 1944.

13. HOWARD, B. J. *Outlines for Instruction in Tomato Microanalytical Methods.* U. S. Department of Agriculture, Food and Drug Administration. Revised December 1, 1937.

14. RAMSEY, GLEN B., JAMES S. WIANT and LACY P. MCCOLLOCH. Market Diseases of Tomatoes, Peppers and Eggplants. U. S. Department of Agriculture. Agriculture Handbook No. 28. Washington, D. C. June 1952.

15. Lighting for Canneries 1950. Prepared by the Sub-Committee on Lighting Study Projects in Industry of the Illuminating Engineering Society. (Reprinted from the January, 1950 issue of Illuminating Engineering.)

16. WILDMAN, J. D. and P. B. CLARK. Some Examples of the Occurrence of Machinery Slime in Canning Factories. Journal of the Association of Official Agricultural Chemists. 30:3, 582-585. 1947.

17. Trade Press Memorandum, U. S. [illegible] and Drug Administration. [illegible] 1951.

18. Statements of General Policy of [illegible] pretation. Comminuted Tomato [illegible] and Containing Excess Natural [illegible] and Regions, July 20, 1951 (16 [illegible] 6997). NCA Information Lett[er] 1340, 278. July 21, 1951.

19. National Canners Association [illegible] published report.) 1953.

20. TROY, V. S. *Responsibility for [illegible] of Mold Count of Tomato [illegible]* The Canner. [illegible] Ja[illegible] 1950.

Bibliography

AMERICAN CAN COMPANY. 1954. [illegible] Mold Count Method as App[lied to] Tomato Products. American Can Co[mpany], Research Division, Maywood, Illinois.

BARTA, E. J., and LOWE, F. [illegible] into the Reduction of Mold Count [illegible] Berry Boxes. The Canner. [illegible] 14-15. July 28, 1940.

[illegible] Fred L. Speed Tom[ato] [illegible] with the Hand's [illegible] Engineering 27: No. 10: 112, 150-152. [illegible]ber, 1951.

BURNS, [illegible] E., and D[illegible] Examining Tomatoes for Mold [illegible] Journal Paper No. 715. Agricultur[al Ex]periment Station, Purdue Universi[ty], [illegible], Indiana. 1953.

DOOLITTLE, S. P. Tomato Disease[s] [illegible] Department of Agriculture. Farmers' [Bulle]tin 1934. U. S. Government Printi[ng Of]fice, Washington. Revised March 1[illegible]

[illegible] W. V. Sanitary [illegible] Tomato Product Plants. The Glass [illegible] 31: No. 1: 25-28, 56-57. January [illegible]

[illegible] W. V. Factory Con[trol] [illegible] Tomato Stock for Tomato Products [illegible] Packers. [illegible] No. 1: 38, 40, 48, [illegible] [illegible] 1952.

...ss, W. V. *Factory Control of ... Matter in Tomato Products*. The Canner and Packer. 44: No. 4: ... April 1952.

... Staff. *Lug Boxes with New Resin Coating*. Food ... 21: No. 7: 71-72. July 1949.

... T. 1931. *The Sorting of ... Training of Sorters; Objection... of Tomatoes*. The Canner. ... 1931, p. 11.

...simon H. *The Microscope*, 17th ... New York. Comstock Publishing ..., Inc. 1947.

...an, C. O. 1946. *Quality in ...uice, Part III. Relation of Mold ... to Quality*. Canadian Food ..., No 9, 35. September, 1946.

... Kenton L. *An Annotated ... of Methods for the Exam... Foods for Filth*. Journal of the ...n of Official Agricultural Chem... No. 4: 42[illegible]-439. 1946.

... William C. *Mold Count as ... Quality of Tomato Juice*. [illegible] ... 21 No. 9 [illegible] ...

... [illegible] ... Control. Food Industries ... June 1942.

..., B. J. 1937. *Comments on ...ems of Sorting Tomatoes*. The ... June 19, 1937, pp. 11, 12; The ... Trade, June 21, 1937, pp. 7, 34.

... [illegible] ... Tomato Canning ... U.S. Department of Agriculture ... June 21 1937.

... A. H. and [illegible], Margaret E. ... of Canned Tomato Prod... Food Industries ... February 1947.

... H. and [illegible] W. F. The ... of Canned Tomato Products. ... Food Industries ... December 1945.

Lamb, M. B. and Wright, J. M. *Tomato Diseases and Insect Pests: Identification and Control*. University of Illinois College of Agriculture, Circular 683. Urbana, Illinois. May 1951.

Neubrauch C. W. *Training the Tomato Products Technician. 1. Apparatus for Studying Mold Counting Technique*. The Canner. 99: No. 3: 18-20, 34-36. June 17, 1944. 2. *Handling the Individual Fields*. The Canner. 99: No. 4: 14, 16, 24, 26. June 24, 1944. *Conclusion*. The Canner. 99: No. 5: 14-15, 18. July 1, 1944.

Neubrauch, C. W. *An Assist to the Novice Mold Count Technician*. The Canner. 115: No. 2: July 12, 1952.

Neubrauch, C. W. *Tomato Technician's Manual* (unpublished) 1952.

Pitman, Gilbert A. *Mold Count Recording Device*. Journal of Association of Official Agricultural Chemists. 26: No. 4: 511-513. 1943.

[illegible], Marvin and [illegible], James V. *The Significance of "Mold Counts" in Tomato Products*. The Canning Trade. 72: No. 37. August 8, 1949.

[illegible], Charles E., [illegible], Chester W., and Thompson, Henry W. *Henrici's Molds, Yeasts and Actinomycetes*, 2nd ed. New York, John Wiley & Sons, Inc. 1947.

Smith, Howard R. *Instruction in Microanalytical Methods*. National Canners Association Research Laboratory, Washington, D. C. [illegible] ... at the [illegible] annual meeting of the A.O.A.C. Washington, D. C. [illegible] ... J. Assoc. of Official Agricultural Chemists. [illegible]

Tanner, Fred W. *The Microbiology of Foods*, 2nd ed. Champaign, Illinois. Garrard Press. 1944.

Tanner, Fred W. *Laboratory Manual and Work Book in Microbiology of Foods*. Champaign, Illinois. Garrard Press. 1950.

Troy, V. S. *The Importance of Technician Schools to the Tomato Canner*. The Canner. 112: No. 13: 16-17. March 31, 1951.

MODIFIED SLIDE PREPARATION FOR THE OFFICIAL HOWARD MOLD COUNT METHOD*

By A. A. Kopetz (American Can Co., Maywood, Ill.), V. S. Troy (Continental Can Co., Chicago, Ill.), and M. R. McCallum (National Canners Association, Washington, D. C.)

Experience in performing the official Howard mold count method, **35.58** (*Official Methods of Analysis*, 8th Ed., 1955, pp. 781–782), and in instructing analysts in its use has shown that preparing the slide mount still presents some difficulties, such as uneven distribution of the insoluble solids and entrapment of air bubbles between the central disk and cover glass. To overcome these difficulties, the slide preparation has been modified by substituting careful manipulation of the cover glass for the official pre-spreading technique.

The modification has these advantages: (1) distribution of the insoluble material is consistently uniform; (2) air entrapment is virtually eliminated; (3) the mount can be prepared faster and more easily; and (4) scratching of the glass of the central disk is reduced. Those who have used this modified technique agree that it produces no significant difference in the random distribution of mold filaments, other than the normal variation inherent in sampling a product that is a mixture. Table 1 and Figure 1 show a limited comparison of recent data to support this opinion.

Because the Howard mold count method is empirical, it must be used exactly as stated to obtain accurate results. Although the method has been revised several times since its development in 1910, its fundamental principle has remained unchanged.

The present form of the official method contains several improvements not in the original method (1). These revisions (2–6) have helped to standardize the technique of mold counting and have made it more uniform. We feel that the proposed modification serves this same purpose.

This modification of the preparation of the mold count slide was first used in 1945 by Mrs. Gertrude Kissell, of the National Canners Association Research Laboratory. Analysts who have performed it since that time have used two techniques, both of which eliminate spreading the sample portion over the surface of the central disk with the knife blade or scalpel, as in the official method. However, both techniques retain and utilize the spreading, distributing action that results from lowering the cover glass into place. The two techniques are as follows:

A. INCLINED COVER GLASS TECHNIQUE

Using a spatulate instrument, take a portion of a well-mixed sample and transfer to an area on the central disk half-way between the center of the disk and the far

---

* Presented at the 70th Annual Meeting of the Association of Official Agricultural Chemists, Oct. 15–17, 1956, at Washington, D. C.

TABLE 1.—*Comparison of results of Howard mold counts by official and modified methods for preparing slides*

SAMPLE NUMBER	TOTAL FIELDS COUNTED, EACH METHOD	OFFICIAL METHOD[a]					MODIFIED METHOD[b]				
		POSITIVE FIELDS SHOW				% MOLD COUNT	POSITIVE FIELDS SHOW				% MOLD COUNT
		No. 1	No. 2	No. 3	No. 4		No. 1	No. 2	No. 3	No. 4	
Tomato Juice											
1	50	2	0			4	1	4			10
2	50	0	2			4	2	1			6
3	50	1	3			8	1	2			6
4	50	1	4			10	2	2			8
5	50	6	3			18	6	4			20
6	50	5	7			24	1	7			16
7	50	5	2			10	5	2			14
8	50	2	1			6	3	2			10
9	50	1	1			4	1	0			2
10	50	0	1			2	2	2			8
11	50	4	2			12	4	3			14
12	50	3	3			16	0	2			4
13	50	13	6			38	14	14			56
14	50	4	3			14	6	7			26
15	50	6	3			18	3	2			10
16	50	3	8			22	2	4			12
17	50	2	3			10	2	2			8
18	50	3	6			22	2	3			10
19	50	6	5			22	5	4			18
20	50	3	6			18	3	6			18
21	50	4	6			20	1	7			16
22	50	0	1			2	3	0			6
23	50	4	1			10	3	4			14
24	50	1	1			4	1	0			2
25	50	3	0			6	2	1			6
26	100	10	9	8	11	38	8	10	11	8	38
27	100	3	3	2	3	11	5	1	2	2	10
28	100	4	1	1	3	9	2	2	2	4	10
29	100	1	4	2	2	9	3	4	4	2	13
30	100	0	1	1	0	2	0	1	0	0	1
31	100	0	1	0	0	1	0	0	0	0	0
32	100	1	0	3	2	6	3	0	3	1	7
Catsup											
33	50	8	8			32	7	8			30
34	50	14	14			56	15	15			60
35	100	11	7	9	8	35	8	8	9	9	34
36	100	11	8	9	11	39	12	9	10	11	42
37	100	12	10	9	13	44	12	13	10	12	47
38	100	11	9	10	12	42	13	9	13	11	46

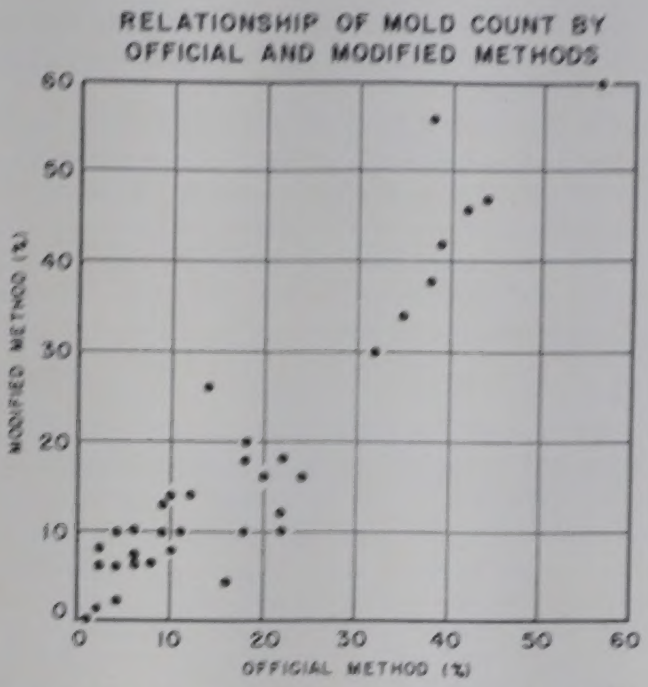


FIG. 1.—Relationship of mold count by official and modified methods.

edge. (A dissecting needle may be used to remove the sample portion from the spatula to the central disk.) Rest one edge of the cover glass in a slanting position on the edges of the slide shoulders nearest the portion of test material. Lower the cover glass slightly until it almost touches the test material on the disk; then lower it into place rapidly, but gently, so that the material spreads evenly over the entire surface of the disk.

B. PARALLEL COVER GLASS TECHNIQUE

Using the transfer method described in technique A, place the sample portion on the approximate center of the central disk. Hold the cover glass parallel to the surface of the central disk and lower it slowly until it just touches the sample portion. While maintaining contact with the test sample, alternately lower and raise the cover glass very slightly two or three times; then without stopping, lower it rapidly but gently until it just touches the shoulders of the slide, so that the test portion spreads evenly over the entire surface of the disk.

DISCUSSION

In both techniques, as in the official method, the cover glass should not be lowered too rapidly or part of the sample may splash over onto one or both shoulders, thus ruining the mount. Neither should it be lowered too slowly, or the insoluble material will not spread uniformly.

With a little practice, this step can soon be controlled so that slides showing evenly distributed insoluble material can be consistently prepared. Although even trained counters have difficulty in preparing slides

by the official (pre-spreading) method, experience shows that the mod
techniques are learned quickly and less difficulty is encountered th
after in slide preparation.

As discussed in the official method, any mount showing uneven distr
tion of insoluble material, absence of Newton's rings, or liquid that
been drawn or splashed across the moat onto the shoulders should be
carded. No slide should be counted unless it is properly prepared.

The authors have had considerable difficulty with the modified meth
as well as with the official method, when 33×28 mm cover glasses[1]
used on the Howard slides constructed with a round central disk. The
fore, square cover glasses (33×33 mm) are recommended for use in
modified method.

If the Association approves, the authors recommend that this modif
tion be incorporated in the official Howard mold count method, 35
as an alternate technique. Only the second paragraph of the pre
method need be changed. The suggested change in wording is as follo

Clean the Howard cell, 35.1(j)(1), so that Newton's rings are produced betw
slide and cover glass. With spatulate instrument place portion of well-mixed sar
upon central disk; prepare mount, using only sufficient sample to bring materi
edge of disk, so that insoluble solids are uniformly distributed in sample on
Mount sample by: (a) using spatulate instrument to spread sample evenly
disk and covering with glass so as to give uniform distribution or (b) lowering c
glass rapidly but gently over sample on disk to give uniform distribution. (It
utmost importance that portion be taken from thoroly mixed sample and distrib
evenly over slide disk, otherwise insoluble material and consequently molds ma
unevenly distributed.) Discard any mount showing uneven distribution or abs
of Newton's rings, or liquid that has been drawn across moat and between c
glass and shoulder.

ACKNOWLEDGMENT

The authors wish to thank W. V. Eisenberg, U. S. Food and Drug
ministration, for his assistance in the formulation of the suggested cha
in wording of the official method.

REFERENCES

(1) Howard, B. J., *Tomato Ketchup under the Microscope with Practical Suggest
to Insure a Cleanly Product*, U. S. Department of Agriculture, Bureau of Ch
istry, Circular No. 68 (out of print), February 13, 1911.
(2) Association of Official Agricultural Chemists, *Report of the Committee on Edi
Tentative and Official Methods of Analysis*, pp. 324–325. Williams and Wil
Co., Baltimore, Md., 1916.
(3) Howard, B. J., and Stephenson, C. H., *Microscopical Studies on Tom
Products*, U. S. Dept. Agriculture Bull. No. 581 (out of print), October 6, 1
(4) Howard, B. J., *This Journal*, **6**, 50 (1922).
(5) *Official Methods of Analysis*, 4th Ed., Association of Official Agricultural Ch
ists, Washington, D. C., 1936.
(6) *Ibid.*, 8th Ed., 1955, pp. 781–782.

[1] Designed for use with counting chamber having a rectangular central mounting area.

Other Bulletins Available

tionship of Spoilage to Rough Handling and minated Cooling Water.

ntion of Vitamin C in Tomato Juice.

tion in the Food Plant.

ct of Radioactive Phosphorus upon a Suspension of richia Coli.

r Spoilage of Tomato Juice.

of Canned Foods.

of Producing Vacuum in Cans.

nstallation, Equipment and Operating Procedures.

Principle for Agitating in Processing of Canned Foods.

or Canning.

Browning and Related Ripening Disorders of toes.

oduct Quality Control.

f Progress in Tin Plate Manufacture on the Use of late for Cans for Foods.

Sterilization Temperatures on Color of Tomato Juice.

ring the Air Removal from Canned Foods.

in Canned Foods—Its Significance ts Measurement.

ention of Carotene During the Canning of to Juice.

nning of Acidified Banana Puree.

Method for the Presumptive Identification of ria which have been associated with off-flavors odors in Concentrated Orange Juice.

Studies of Canned Single Strength Orange Juice.

Blanching of Peas.

Regeneration in High Temperature—Short Time ized Canned Foods.

spects of Canning Soft Drinks.

ing Plantings and Predicting Harvest Maturities rocessing Vegetables.

ngs.

characteristics of spoilage organisms in orange juice oncentrate.